

nature

Volume 248

March 8, 1974

No-one wins

WHAT did the general election in Britain mean—and what does it portend? British readers, worn out with journalistic analyses, will have to bear with us for a week for the sake of our overseas readers.

Hardly anyone in the political world can be very pleased by the present indecision. Mr Wilson heads a Labour Party whose number of seats falls short of the 318 needed for an absolute majority. Mr Heath, having gone to the country early to seek a strong mandate to deal with threats to his counter-inflation policy, found the country unresponsive to him. Mr Thorpe, having done wonders in making a Liberal revival look credible and worth voting for, gets 20% of the popular vote and only 2% of the seats in Parliament.

The campaign has been surprisingly moderate and serious in character. The central issue on which Mr Heath called the election was the deadlock between the government and the miners' union over pay increases, and it is clear that Mr Heath originally hoped that the country would see him as a moderate, fighting off union extremism. His motif has been 'firm but fair', repeated so often and so similar to the motif 'frei aber froh' of Brahms' Third Symphony that one was surprised Mr Heath, a cultured man, did not use the opening of that symphony to herald his electioneering appearances. Words become codewords in elections and 'firm' clearly was meant to signify 'tough on the unions' so here was an opportunity for a bitter, divisive election reviving all the class conflicts that periodically wrack the country. That this did not happen appears to have been due to two factors: the two major parties perceived that a doctrinaire battle would have allowed the Liberals to take the middle ground where the real majority always lies, and Labour was able to broaden electoral issues to the whole economic scene. Neither major party has been very explicit about what it would do for the miners, but it could be inferred that whatever the result, miners would get substantial raises, through some quasi-judicial process from Mr Heath or through some great emotional gesture from Mr Wilson.

What do the results portend?

The parliamentary system is about as close to deadlock as possible. Suddenly the much vaunted British system for maintaining two party democracy has creaked, for no leader can now ignore the Liberals and other parties which have led a shadowy existence for so many years. Liberals and Nationalists are at least relatively attractive partners for a coalition, less so the eleven Protestant Ulstermen dedicated to bringing down the tentative attempts at cooperative democracy in Northern Ireland.

Unfortunately, the national skill for compromise is poorly exercised in putting together coalitions. Labour

in particular still has bitter memories of the thirties when a coalition nearly annihilated the party. Both major parties have been hurt by the swing to the Liberals, but Mr Wilson can soldier on for maybe a year or more with the grudging support of the Liberals and Nationalists, and then turn to the electorate with a good chance of gaining an absolute majority. This Mr Heath must have perceived for he initially refused to resign and has attempted to put together a government of his own by courting the Liberals.

Much, however, must now change politically. The Liberals could well force electoral reform. The Nationalists could well force devolution of power on many issues to regional parliaments. Most important, if the grip of the two major parties, both so clearly identified with class distinction, can be loosened, it is possible that in the next fifty years Britain, still grossly class ridden, can develop a more equitable society. Neither party is able to achieve that at present.

100 years ago



In a recent number we intimated that the Perthshire Society of Natural Science had interrogated the Parliamentary candidates for the county and city of Perth as to their opinions on the questions of State help to Science, a responsible Minister of Education, and the promotion of Scientific Exploring expeditions.

Answers—favourable, we are glad to say—were returned at the time only by the two Conservative candidates, one of whom, Sir W. Stirling Maxwell, is now M.P. for Perthshire. We are now glad to give place to the somewhat tardy reply, addressed to the secretary, of the Hon. Arthur Kinnaid, Member for the City of Perth:—"I, Pall Mall East, 18th Feb. 1874.—Dear Sir,—I was surprised to find copied into a London paper from a Scotch journal the questions put in your letter of the 29th January last, with the statement that they had not been answered by me. The fact of my being, as I believe I am, one of the patrons of the Perthshire Society of Natural Science should have been, it appears to me, a sufficient guarantee of my approval of the objects of your institution; and my active co-operation with Capt. Wells in his efforts during the last session of Parliament to obtain the sanction of Government to a proposed grant for the furtherance of Arctic exploration, further approves my appreciation of the objects you advocate, in my willingness to support State expenditure for well-devised schemes of scientific research and educational purposes.—Yours truly, A. Kinnaid."

From *Nature*, 9, 350, March 12, 1874.



Decisions about the European computer industry

In deciding whether to approve more funds for Compagnie Internationale pour l'Informatique (CII), the French member of the European computer grouping Unidata, the French government is having to weigh up the long term prospects of, for example, an alliance with an American company. Professor A. S. Douglas, Professor of Computational Methods at the London School of Economics and Past President of the British Computer Society examines the underlying issues.

EUROPEAN cooperation, as has been recently demonstrated in Washington, is often difficult to achieve. Nowhere had this proved more true than in computing, and the latest report of the problems of CII is only one further manifestation of the phenomenon.

Case for indigenous industry

There are some very obvious reasons why an indigenous computer industry is desirable in any country. Industry, commerce and government itself are all coming increasingly to depend on the efficiency and speed of computers in handling clerical tasks, plant control and planning studies. The industry is already the third largest, in terms of turnover, after oil and aircraft. At least one company in the industry, IBM, is highly profitable. Thus each government, nervous of the effect which foreign control of this key resource might have on its own efficiency and that of local industry, seeks to protect itself and its 'charges' as best it can, and local interests seek to participate in the profits which should flow from a new and growing industry.

Since the computer industry has its roots, in the main, in the United States, the general fear of external interference may be made more or less pointed by the political climate existing between any country and the United States. The more concern over political implications, the more urgency is there to avoid undue dependence on technology from the United States; the stronger the local interests in the industry, the more concern there is to build up local industry under some form of protective umbrella which will hamper United States activity locally.

The arguments which have led many governments, including those of Britain, France, West Germany and Japan, to subsidise and protect their local interests against the supposed 'threat', are, of course, valid concerning neighbouring governments which may be thought to hold an important card in the industry. Thus cooperation is apt to be approached in a spirit of suspicion. Only if the external threat to a group is deemed strong enough, or if there is an obvious pay-off for all, is it likely that the suspicions will be overcome and a real measure of cooperation take place.

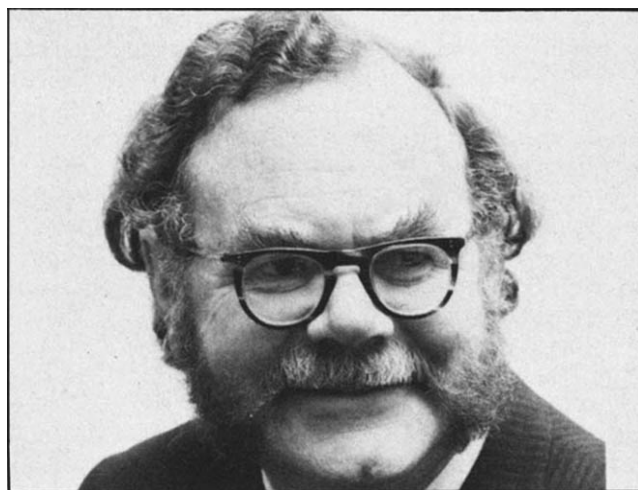
Arguments for cooperation

Setting aside the strength (or otherwise) of this 'threat' from the United States—on which opinions will clearly differ—and the rather more general sentimental arguments in favour of cooperation within Europe, the primary arguments for cooperation are commercial. Computers, even minicomputers, are relatively complicated machines, both to

build and to maintain. Good design makes for reliability and ease of maintenance but such design only results from the incorporation of experience. Experience is gained from use, and is the more quickly learned the more machines are sold and used. Thus quantity production leads not only to reduced unit costs, as with all products, but also to a more sharply convergent 'learning curve' towards the lower unit costs themselves.

There is, then, a double prize for achieving quantity production, and this can only be done in a large market. Europe is a fast-growing computer market and will, no doubt, in time become similar in size to that in the United States. A truly European marketing strategy, if pursued without political hampering, ought to be sound commercially. A sensible selection of points of attack on the dominance of the United States ought to succeed, whether or not governmental support is available, provided the companies concerned are at least as well managed as their competitors. All companies are, however, somewhat vulnerable in the course of their early developments and when a major investment in say, a new range of computers is being made, so perhaps there is a case for support. What is really needed is access to the whole European market on a basis of parity in each country and the price for this seems to be 'multinationalisation' of the firms concerned.

Because I believe that there is sound commercial sense in aiming to achieve a freer market situation, I would suppose that governments will press forward with collaboration—though obviously on the best terms each feels it can get for its own protégées. Probably, therefore, a solution will be found to the problems of CII and cooperation with Siemens and Philips will continue.



Professor A. S. Douglas

Britain

Meanwhile the British position requires careful consideration to see what is likely to be in the interest of British industry as a whole. Clearly ICL is an important part of the industry and its interests must be considered. But there are important sections in peripherals, minicomputer terminals, software and bureau operation, where government action can have equally important influences and where money is to be made both at home and abroad by good husbandry. So far, the Department of Trade and Industry has tended to pay very little attention to these areas and has concentrated its policy around the support of ICL alone. Recently there have been welcome signs of change, and it is to be hoped that a broader view will be taken in the future.

international news

Behaviour modification in the limelight

Colin Norman, Washington

IN the past few weeks, the topic of behaviour modification has been accorded considerable public attention and a good deal of newsprint in the United States. The sudden burst of publicity stems from two separate decisions by government agencies to withdraw support from potentially embarrassing projects, and the affair bears witness to the strong and growing public concern about research projects involving human experimentation.

The first government action to arouse interest was a decision by the US Bureau of Prisons to terminate a controversial project at a federal psychiatric prison at Springfield, Missouri. Called Start (an acronym for special treatment and rehabilitation training), the project involved rewarding good behaviour by granting so-called privileges to the inmates. The project was terminated, according to the Bureau of Prisons, because it was too costly, but the decision came after a group of inmates had filed suit in the federal courts, contending that Start violated their constitutional rights because it constitutes cruel and unusual punishment.

The second, and perhaps more important, episode was an abrupt decision by the Law Enforcement Assistance Administration (LEAA) to withdraw funds from projects involving "psycho-surgery, behaviour modification—including aversion therapy—and chemotherapy". According to LEAA Administrator Donald E. Santarelli, the decision was taken because the agency lacks the skills to monitor such research, and in any case, the projects are peripheral to LEAA's business of providing assistance to state and local law enforcement activities.

Those two incidents seem to indicate that the Nixon Administration has taken a critical look through its programmes involving human experimentation and has decided to weed out the more questionable among them. Unfortunately, however, that is not the

case and the two actions by themselves clear up none of the questions about the acceptability of behaviour modification, nor do they indicate any new policy throughout the Administration on human experimentation.

For one thing, the debate has so far been completely unencumbered by a definition of what constitutes behaviour modification, and for another, the two decisions were taken for bureaucratic, as opposed to ethical, reasons. But the actions will have beneficial consequences, and if nothing else, the public attention indicates that the concern over safeguards on human experimentation—a concern which owes much to the revelation in 1972 that a federally sponsored research project had denied treatment to a number of poor black men who were known to have syphilis—is making it much more difficult for questionable projects to carry on unnoticed.

As for the LEAA decision, Santarelli announced that his agency is getting out of the business of medical research and behaviour modification entirely, and that proposals for funding such research in future will simply be referred to the Department of Health, Education and Welfare. The agency, he said, is now conducting a search of the projects it has been funding, to see which fall into the forbidden category.

Given those statements, it may reasonably be asked what controls the agency has been applying to safeguard the rights of subjects in research projects it has been funding. The answer is pretty obvious from the fact that the agency does not even know what research projects it has been supporting, although its own guidelines dictate that each project involving medical research "should receive individual and prior approval by LEAA". Most LEAA money is provided in the form of block grants to state and local governments, and thus the agency is not always fully aware of every individual project receiving its support.

Without a definition of what constitutes behaviour modification, it is difficult to know exactly what LEAA intends to scrap, but some of its projects are clearly in jeopardy. It has been funding a project in Puerto Rico, for example, which involves the use of drugs in an attempt to see whether there is a link between criminality and brain damage. The agency has also

been supporting a project in Pennsylvania which is designed to test B. F. Skinner's theory of reward conditioning in the treatment of young offenders. Those projects will clearly lose their LEAA funds, but there is a whole grey area of projects that cannot easily be categorised, and whose future is therefore uncertain.

LEAA's decision is also likely to have hammered a nail into the coffin of a controversial proposal to establish a Center for the Study and Reduction of Violence at the University of California, Los Angeles. The centre which was proposed in 1972 and which was embraced by Governor Ronald Reagan in his State of the State message in



LEAA Administrator Donald E. Santarelli

January 1973, had applied for a \$750,000 block grant from LEAA, and for a similar amount from the State of California. LEAA had already consulted HEW on the matter, but it will now wash its hands of the proposal and the ultimate decision on whether to fund the centre will probably rest with HEW.

The centre has already caused a huge amount of controversy at UCLA and it has come to national attention largely through the work of a Washington-based public interest group called the Children's Defense Fund. Proposals for the centre, which have undergone many revisions since they were first put

forward in 1972, include investigations of the use of cyproterone acetate, a controversial so-called castration drug, on sex offenders; investigations designed to determine whether there is any correlation between abnormal electrical activity in the brain and violent behaviour; studies looking for correlation between criminal behaviour and the possession of XYY chromosomes; and biofeedback and aversion therapy for sex offenders. The centre's plans, which have been drawn up by its proposed director, Dr Louis Jolyon West, have been strongly criticised, by both public interest groups and by individuals who have examined them. Given the publicity that has already been attracted by the centre, it seems unlikely that it will be able to get through the network of review committees that HEW requires for research involving human subjects.

HEW, in fact, now has under consideration a proposal for strengthening its control of human experimentation (see *Nature*, 246, 239; 1973), and the Congress is considering a bill to strengthen the rules even further. Thus the LEAA decision will have the effect of shifting research into an agency where it will be reviewed rather critically on ethical and legal grounds.

As for the decision of the Bureau of Prisons to scrap the Start programme, that action is less far reaching, but nevertheless it is an important indicator that even the officials who operate the federal prison system, who have been heavily criticised by prisoners' rights organisations and other groups, are not unaware of the embarrassment potential of questionable human experimentation.

Last week, in fact, Mr Norman Carlson, Director of the Bureau of Prisons, and Dr Martin G. Groder, the Warden-designate of a medical research prison being built at Butner, North Carolina, were called before a committee of the House of Representatives to explain the bureau's behaviour modification studies. Both men denied that any aversion therapy is being carried out in federal prisons, and they agreed that the Start programme had some faults, chief of which is that the prisoners selected to take part had no choice in the matter, thereby violating one of the chief safeguards in any human experimentation: informed consent.

The committee was anxious to know about the plans for the Butner facility, and about the safeguards that the bureau will provide to ensure that research carried out there does not violate prisoners' rights. According to Groder, the prison, which will be capable of housing up to 340 prisoners, will be partly a medical treatment facility for prisoners with severe mental disorders, and partly a correctional research facility. As far as the

latter function is concerned, it will test various psychological and therapeutic methods of behaviour control, such as group therapy on the Synanon drug treatment model, and those that are found to be successful will be applied throughout the prison system.

Groder specifically stated that "none of the methods (of behaviour control) already preliminarily chosen or being considered favourably, involve the methods of modern-day torture known as aversive conditioning, specifically the misuse of drugs, electric shock or psychosurgery". That was greeted with favour by the committee, but the Congressmen were less impressed with the proposed methods for ensuring that prisoners taking part in the research programmes do so entirely of their own accord.

Groder made clear that those who will take part will have every opportunity to decline to take part, or opt out once the programme has started. But he also said that the prisoners selected to have a chance of taking part will all be within a few months of being considered for parole. At that stage of his incarceration, a prisoner is likely to do whatever he can to help assure his release—including taking part in a research project.

Nevertheless, the upsurge of public interest in safeguarding subjects of human experimentation should ensure that the Butner facility is kept under close and critical surveillance.

Vets make pay claim

John Hall

FIVE hundred veterinary surgeons employed by government departments in Britain have started a work to rule in support of a pay claim calculated to be in the region of 20%. The claim is vaguely defined and long delayed because the pay of veterinary officers is tied to that of scientists in government service, who are themselves waiting for action on a protracted and bitterly pursued claim. In hard cash terms, however, the vets are clear about their long term aims: an increase of £900 to £1,000 a year in the pay of grade one veterinary officers, currently earning £2,996 to £4,004.

Their industrial action threatened the government's brucellosis eradication programme and day-to-day essential work like the implementation of food orders and quarantine supervision. They gave a specific undertaking that they would not halt any work whose interruption would cause a health hazard,

either to animals or humans: the last time they threatened industrial action in similar terms, swine vesicular disease broke out the next day and their disruptive plan was called off.

Announcing the work to rule, the vets' union, the Institution of Professional Civil Servants (IPCS), painted a picture of a sadly declining profession, manned by middle-aged gents whose retirement will leave the service in a state of virtual collapse. Mr Cyril Cooper, Deputy General Secretary of the IPCS, said the service is operating on a manpower 30% below the required complement and that in the next eight or nine years something like half of the vets in government service would have reached retirement age, leaving the department in a "disastrous" situation.

Recruitment has almost come to a standstill because of the disparity between the salaries of vets employed by the state and those in private practice. In 1972, 300 graduates entered private practice, compared with eight entering government service, and of the 8,500 vets at work in Britain the state service accounts for a diminishing group of less than 5%. Although an arbitration tribunal in 1968 expressed doubts that a cash settlement would improve recruitment, and a more recent working party suggested that changes in the structure of the profession might offer a solution, the vets themselves have no doubt that the disastrous recruitment figures can only be reversed by a salary review on a grand scale.

They point out that since the beginning of 1971, the cost of living index has risen 28%, and wages generally have risen by 41%. Their own salaries have risen by between 19.5% and 26.5% depending on grading and so, in order to bring state vets back to their former position, a relativity increase of about 20% is needed. At present, salaries paid to the three grades of Government Veterinary Officer are: grade one, £2,995 to £4,004; grade two, £2,616 to £3,312; and Divisional Veterinary Officers, £4,240 to £5,912. They suggest that their future salaries should be compared with those of doctors, who can expect between £6,700 and £7,200 a year, and with dentists, who may be £600 below that level.

The Veterinary Service, they say, has operated for years on the good will of vets who were willing to cut corners and do work which they were not strictly required to do. But the goodwill is now in scarce supply, and since governments of different political complexion have shown themselves to be equally bad as employers, the vets thought they could only make their feelings known by applying pressure based on industrial action. They will do no overtime, give no lectures outside working

hours, take no work home, take time off in lieu of any weekend work they have to do, refuse to use their home telephones for official calls and stop doing jobs which ought properly to be done by an officer of a higher grade. Unless there's an outbreak of foot and mouth, that is.

Soviet breeder reactor accident

from our Soviet Correspondent

RECENT unofficial reports, apparently based on data from United States satellites, indicate a serious accident to the Soviet fast breeder reactor BN-350, which formed the heart of the new town of Shevchenko on the Mangyshlake Peninsular on the Caspian Sea.

The reactor was designed to power a desalination plant producing more than 100,000 cubic metres of fresh water a day and was intended to transform the desert shore of the Caspian "where even the camels look weary" into a modern, industrial model township with a population of 80,000, complete with parks, fountains and avenues of shady trees. The town was clearly intended to be a showpiece, and was accordingly named after the nineteenth century Ukrainian poet Taras Shevchenko, although the "drowsy waves, sky unwashed and dirty" and the "worthless sea" to which he was exiled was not in fact the Caspian but the Aral. Shevchenko was, however, a figure of world renown (even meriting a statue in Washington DC) and was considered a suitable eponymous hero for what was intended to become a showpiece of national and international importance.

Reports of the disaster pose some interesting problems when compared with the official publicity handouts. According to the Novosti Agency, the reactor, which was commissioned on September 27, 1972, was officially "launched" on July 16, 1973. Shortly afterwards an *Izvestia* correspondent visited the station and described in glowing terms the reactor with its control centre "like the bridge of an imaginary spacecraft". An influx of visitors was clearly expected—a special display showing a mockup of one of the fuel elements was exhibited in the entry hall. Even the *Izvestia* correspondent, however, noted the hazards of the "exotic coolant", molten sodium: but he stressed that special safety measures had been incorporated in the design.

The BN-350 was designed to operate on the three-circuit principle: in the first circuit the sodium flows directly round the fuel elements (steel "pencils" containing 6 mm pellets of uranium-235-

enriched uranium) and itself becomes radioactive, giving off heat through the steel heat exchanger walls to the molten sodium and the second circuit which is heated to 450° C. The sodium in the second circuit then heats the water in the third circuit in the usual way, producing a nominal 1400 tonnes of steam an hour. In all, the reactor has six such three-circuit loops. The total thermal power of the reactor is rated at 1 million kW (1GW) which can be divided between electricity generation and desalination as required. Initially, an optimal regime of 150,000 kW of electricity and 12,000 tonnes of desalinated water a day was envisaged.

The reports and rumours of damage suggest that something has failed in the cooling channels—though whether this refers to the first or second sodium circuit is not definite. Clearly, either could cause the "large fire" which the satellite data are said to indicate: damage to the first circuit, however, would be far more serious, involving a considerably greater radiation hazard.

The most curious feature is the date of the alleged disaster. That generally

mentioned is July 1973, shortly after the official "launching". But as late as August 21, 1973 the Novosti agency issued a three-page report on the BN-350, the "atomic heart" of the Mangyshlake Peninsular (a length of coverage normally given only to matters of major international importance). Either the satellite dating is incorrect, or the Novosti agency was particularly late in releasing information, or some attempt was made at a cover-up operation—a not unlikely contingency, in view of the prestige attached to developments at Shevchenko.

Chemists think about energy

IN Britain a group of top university chemists has been talking with scientists from Imperial Chemical Industries, British Petroleum and the Department of Energy in order to define long term prospects for evolving alternatives to fossil fuel supplies. The meeting followed an initiative by Professor R. Mason, Chairman of the Science Board of the Science Research Council (SRC), and among the academics brought in for the talks were Sir George Porter, Professor Sir Derek Barton and Professor Geoffrey Wilkinson. Similar discussions are likely to take place in other departments of the SRC, and a joint communique is expected in a month or so. In the meantime the Chemistry Study Group has issued the following statement:

"The Science Research Council has a broad responsibility for promoting research of 'timeliness and promise' which, together with its involvement in postgraduate education, can be expected to provide advances in fundamental scientific knowledge. However, within this general remit, the council is concerned to assess the balance of its funding pattern in relation to national needs or priorities. In common with many other scientific research funding agencies throughout the world, events of the past three months have caused it to take a new look at topics of research which may have a bearing on the energy situation.

A Chemistry Study Group of the Science Board (Secretary, Dr S. M. Mellows) has recently debated preliminary views of possible chemical innovation which might be applicable in the general context of energy supply. The Study Group accepted that any short term solutions to the problems in this area will most probably be based in industry, or, say, the Department of Energy rather than in the universities or polytechnics and, therefore, concentrated on longer term prospects such as the need for synthetic fuels in the year

Switched off in academe

A SUGGESTION that universities might lose their exemption from the working of the three-day week in Britain has been made in a memorandum issued to heads of departments and administrators at the University of Birmingham. Asking that the use of academic buildings in the evening and at night should be discouraged, the university's Estates and Buildings Officer, Mr J. H. Fathers, told members of staff that he was making the request as a result of an urgent message from the University Grants Committee (UGC).

"On Monday morning" the memo reads, "I received an urgent telephone call from the UGC telling me that the Department of Energy had contacted them to say that representations had been made about the excessive use of electricity in educational establishments. The department inferred that if this continued universities might be specifically placed under the regulations of the three day-week Statutory Order".

The UGC said it had no knowledge of an urgent telephone call and the Department of Energy denied making any inference that universities might be subject to the Statutory Order.

2000. Contributions to the Study Group's discussion from the wider chemical community would be welcomed.

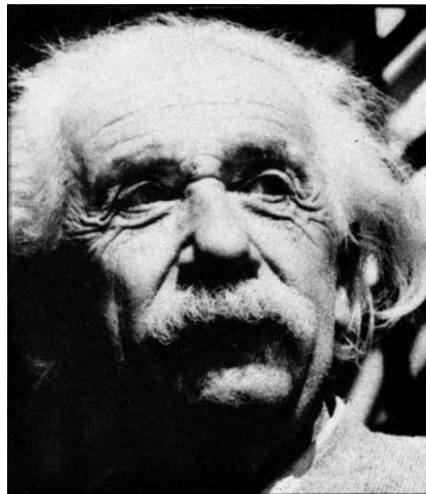
The Study Group's first concern was to assess the possibilities for the fixation of atmospheric carbon dioxide. There was general agreement that emphasis on a biological photosynthesis would be justified, the photochemical synthesis, perhaps metal-catalysed, of organic molecules such as methanol or formic acid appearing to be a challenging and reasonable objective. Indeed much more knowledge generally of the photochemistry of related simple systems, such as the carbonate ion, is needed. The organometallic chemistry of carbon dioxide was discussed at some length and it was recognised that there had been little work directly related to this area; the Study Group's view might be summarised by saying that, although much would accrue in the general field of synthesis, it was not obvious at this time that homogeneous catalytic fixation of carbon dioxide was either technically feasible or economically viable. New developments of heterogeneous catalytic alloys could aid the separation and fixation of atmospheric carbon dioxide but an alternative technology based on molecular sieves appeared to be economically unattractive. It appeared that a single step conversion of methane to higher hydrocarbons or to higher alcohols would be of considerable interest to industry in the short term.

It was accepted that the further development of photovoltaic and photogalvanic cells should be of high priority and that the latter have potential for energy storage. The development of efficient fuel cells, similarly, is of importance but, in view of the heavy industrial investment in this area, SRC's interest should be restricted to well-defined new ideas such as alternatives to platinum electrodes. The low (about 80° C) temperature storage of hydrogen by both organic and inorganic systems emerged from the discussion as a promising area of enquiry while coordination of organic synthetic methods with solid state physics could provide new approaches to the development of the very elusive high temperature superconductor.

The Study Group emphasised that overdependence on nuclear energy for future needs, without heavy investment in research devoted to the utilisation of solar energy, could be hazardous. Of course, heavy investment in new research in these areas will require examination of the technological economics in relation to possible alternative methods. The Study Group will meet again to review any changes in the pattern of research activity and development which might originate from the chemical community as a whole."

Physicist, heal thyself

Michael Stone, East Berlin



Albert Einstein

THE mind boggles at the idea of an opera about somebody as unobtrusive and, generally, devoid of drama as Albert Einstein. The man acknowledged as this century's greatest scientist may have wrought a revolution in modern physics and astronomy, he may have shared responsibility for the development of nuclear research, but his own life was singularly free of anything approaching the sensational. His only personal experience of something nasty happened in 1933, when his books were among those burnt on Goebbels's orders and some Nazis wrecked his study in Berlin. Soon after, he emigrated to the United States where at Princeton University, he continued his studies. In August 1939 he wrote his famous letter to President Roosevelt which is said to have initiated the American nuclear research programme.

In East Germany, where "Einstein" has just had its world première at the Deutsche Staatsoper, mathematics are regarded as almost an Olympic discipline, with annual regional and national competitions, culminating in mathematical championships at different age levels throughout the Socialist countries. Fortunately for the rest of us Paul Dessau and his librettist, Karl Mickel, were more interested in the conscience of Albert Einstein and the success or failure of his endeavours as a pacifist and humanist. Dessau—for many years Bertolt Brecht's closest musical collaborator and friend—combined this subject with an ambition to revive the traditions of the popular musical theatre, from which have sprung such comic figures as the Shakespearean

fool, the harlequin, and Papageno. So he embedded the three acts showing up the relationship between politics and science—first in Nazi Germany, later in the United States—in a comic opera frame, with a prologue, two intermezzos and an epilogue.

Hans Wurst, a traditional comic character in Germany, makes a brief appearance to introduce the opera's basic idea: since science depends upon those in power, scientists ought to develop a social consciousness to make sure that their work serves humanity. In the first entracte, Hans Wurst, (which means as much as Jack Sausage) is thrown to a huge crocodile, symbolising Nazi Germany, but manages to save himself by the expedient of telling the beast a joke until outsize tears of laughter roll out of its eyes. The same trick does not seem to work during the second intermezzo—the crocodile now stands for the America of President Truman and Senator McCarthy—and he is gobbled up. In the end, a resurrected Hans Wurst balances delicately along the edge of an enormous razor, telling us how much he enjoys being alive.

In between such shenanigans we are treated to a semi-historical essay on the role of Einstein and other scientists under Hitler and in the US, the conclusion of which is that Einstein destroys his latest findings to prevent them being misused as, in his opinion, the discovery of nuclear fission had been perverted to blot out Hiroshima.

If this sounds like dry stuff, it isn't. Dessau is a fox who knows exactly how to garnish his score with musical titbits—here a melodious quote from Bach's Toccata, there a series of percussion effects—so that the ear craves for more. The singing is recitative rather than arioso, the orchestra relies more on the brass and the woodwinds, while the strings function mainly in parody; another pointer to Dessau's critical detachment towards the hero of his opera: Einstein was a lover of the violin.

In her production, Ruth Berghaus's scenic humour—she is Paul Dessau's wife and succeeded Helene Weigel as head of the Berliner Ensemble—well matches her husband's playfulness. In the sets of Andreas Reinhardt she provided Theo Adam (looking quite astonishingly like Einstein himself), Peter Schreier, and Reiner Süss (as two other physicists, one of whom makes his peace with the Nazis, while the other lands in gaol) with ever new stage arrangements, ensuring a pace and a high level of acting.

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A silent summer in Washington State

Colin Norman, Washington

THE US Environmental Protection Agency (EPA) has given the go-ahead for what could be the most extensive use of DDT in the United States since the pesticide was banned in 1972. After much soul searching, EPA Administrator Russell E. Train announced last week that DDT can be used this summer to control an infestation of Douglas fir tussock moths, whose larvae have already munched their way through nearly a million acres of fir forests in the Pacific northwest. Although Train said that his decision was reached "reluctantly", he had little choice in the matter.

The moth, which is endemic to the forests of Washington State, Oregon and Idaho, suddenly began a population boom in 1970, and has been eating up an increasing area of foliage ever since. Such infestations usually end in the third year from natural causes, chief of which is a virus infection which spreads through the eggs in the winter and kills off the larvae when they hatch in the summer.

But this population boom failed to follow the three-year pattern, and so timber producers, farmers and the US Forest Service (part of the Department of Agriculture), petitioned the EPA to lift the ban on DDT in order to spray the larvae when they hatch in late May and early June.

The agency was forced to capitulate to demands for DDT for a variety of reasons. First, it refused to accept a similar petition last year on the grounds that the infestation would be wiped out by natural causes, without any help from pesticides. When several hundred acres of denuded trees starkly underlined the moth's refusal to follow past trends, the EPA was made to feel rather embarrassed. Another unfulfilled prophecy this year would be even more embarrassing and would add considerable impetus to attempts by a few Congressmen to strip some authority from the EPA.

Second, there are no alternative pesticides on the market which are known to be as effective as DDT in controlling the moth—a fact which Train last week blamed on the Forest Service because its research and testing programmes "have been almost totally inadequate—to the point of dereliction". And finally, the EPA was under court order to make a decision on DDT use by March 1, which is too early to tell whether there is indeed any chance of a virus infection killing off the pest this year.

The EPA was, of course, reluctant to allow use of DDT on such a large scale because it does not want to open the floodgates to pleas for special treatment for other pest infestations. Thus Train made clear last week that "my approval of this contingency request should in no way be viewed as a departure from the principles of EPA's June, 1972 order cancelling most uses of DDT. I remain personally convinced that the use of DDT represents a significant risk to ecological systems and that its use should be avoided wherever possible".

Train says that he has, in fact, extracted a promise from the Forest Service that DDT will only be used if necessary. In other words, if laboratory and field studies indicate that the population will be wiped out in some areas from virus infection, then the DDT cannisters will remain unopened. The first indications of how the virus infection is progressing will come in the spring when egg masses collected last December are hatched in the laboratory.

If it turns out that DDT will be needed—and it can safely be assumed that the Forest Service will leave little to chance—then the EPA has laid down some fairly stringent rules. Spraying will begin when the eggs begin to hatch in late May, and end by the beginning of July. The pesticide will be applied by helicopter, but an unsprayed buffer strip must be maintained along streams and rivers, no spraying can take place in winds greater than 6 miles an hour and public warnings must be placed in all areas to be sprayed.

Apart from being somewhat embarrassed that their predictions of an imminent collapse in the tussock moth population failed to materialise last year, some EPA officials are also annoyed that DDT spraying has become necessary at all. One official pointed out last week that although the US Department of Agriculture itself banned use of DDT on forested areas in 1968, the department has failed to sponsor an adequate programme to develop alternative pesticides. It was only last summer, in fact, that large-scale field tests of alternatives were conducted, and even then no comparative tests with DDT were conducted. Consequently, EPA has no statistical evidence on the efficacy of DDT.

Train has therefore required, as a condition for approval of DDT use this year, that the Forest Service must undertake a "fully funded, comprehensive research effort on tussock moth control which will support registration of effective and environmentally acceptable alternatives to DDT next year".

Medical museum to move?

Fiona Selkirk

EXHIBITS from the Wellcome Trust's History of Medicine Museum may soon find a new and permanent home in the Science Museum. The Wellcome collection, assembled by Sir Henry Wellcome, contains many fine examples illustrating medical and social history, some early microscopes of excellent quality, fearsome yet interesting surgical instruments and about 200 pestles and mortars, in addition to Sir Henry's more personal collection of horse brasses and the like. The vast medical collection is very valuable and well worth exhibiting.

For many years the Wellcome Trust has been reorganising the museum and the library associated with it, and it has finally come up with a proposal which, with approval from the Department of Education and Science (DES), will mean the wholesale removal of the museum collection, but not the library, to the care of the Science Museum. (Specimens from the History of Medicine Museum can be seen in a current exhibition at the Science Museum, and in July more exhibits will be included in a display commemorating the bicentenary of the discovery of oxygen.)

Unfortunately, it seems that the Science Museum will have to do what the Wellcome Trust is forced to do at the moment—store between 85% and 90% of the collection—but if the proposal is accepted and all goes according to plan, by the end of 1976 there will be a gallery with 10,000 square feet of floor space, for a permanent exhibition of medical history.

At present, the students of medical history who use the facilities of the Wellcome museum and library have full access to the museum specimens in store. A spokesman for the Science Museum said that if all goes ahead, it is hoped to provide the same, and possibly even better, facilities—although, of course, without immediate access to the library.

The DES, which will have to approve the plans for financial reasons, has been kept informed but there have been no definite talks about the final proposal yet.

The Wellcome Trust awards grants for scientific, medical and clinical research, primarily in areas which do not receive large amounts of money from such grant-awarding bodies as the Medical and Science Research Councils. Of a total of nearly £2 million allocated in grants in 1972–73, about £350,000 (18%) went to history of medicine, but the trustees thought that no more

than £250,000 of that allocation could be spent on the museum and library. The target for 1973-74 is £365,000, of which about £90,000 will go in grants and to the units for the history of medicine at Oxford, Cambridge and University College London. It seems that the advisory panel feels that as the trustees wish to devote more funds in future to the promotion of study in the history of medicine the remaining £275,000 this year, and, possibly relatively less subsequently, will not be sufficient to finance the upkeep and development of both museum and library. The panel thinks it is important to develop the latter as a major source of information, not only about medical history but also about more modern medicine.

Museum collections have to be catalogued, maintained and well exhibited, all of which require time, effort and money. The trustees obviously believe that they cannot provide these and so justify the permanent loan of their collection to the Science Museum. There, they feel, the collection can have the care and attention it deserves.

This may be so, but the transfer of what was part of a charitable institution to the care of one funded by public money, regardless of the initial financial assistance proposed by the trust, must come under close DES scrutiny, especially when the time comes that the Science Museum needs more space for its total, ever increasing collection.

Cooperation in coal research

John Wilson

DISCUSSIONS now in progress between the National Coal Board (NCB) and the Office of Coal Research in Washington should lead to closer collaboration between Britain and the United States on research into better ways of using coal. Mr Leslie Grainger, NCB Member for Science, says that an agreement may soon be reached which will allow British access to the results of American research.

Naturally, the NCB's position at these discussions would be greatly strengthened if it were undertaking a substantial development programme of its own. The Board needs to set up pilot plants to test processes already studied in the laboratory and if this were done Britain could form a very useful partnership with the United States.

Formal proposals for such development work were to have been made to Lord Carrington, Secretary of State for Energy, but they have been delayed by the general election. The arguments in

favour of setting up the pilot plants are so strong, however, that they will probably be accepted by whichever party is returned to power.

The urgency with which Mr Grainger views these development plans may be judged from the massive increase in the money he thinks they deserve. In November last year he felt that about £20 million would be sufficient for the next five years. Now, barely three months later, he believes that perhaps twice that amount should be spent. That is the sort of money, he says, which must be made available—and quickly—if British industry is to compete in this new technology by the mid 1980s; the Office of Coal Research has already received \$400 million.

The processes which the board wants to see developed to the pilot plant stage include the gasification, liquefaction and fluidised bed combustion of the coal. It has already carried out much of the preliminary research on these projects and American interests have used its facilities. The Environmental Protection Agency, for example, has successfully burnt American coals in a fluidised bed combustion system at the NCB's Stoke Orchard Laboratories, Cheltenham, in some cases paying entirely for specific tests.

The Office of Coal Research, too, has been studying fluidised bed combustion systems at Leatherhead, Surrey, in conjunction with British Petroleum. It hopes to use the knowledge gained here to build a pilot plant at some 30 MW in the United States. The board would like to match this effort with a similar scheme of their own, possibly at Grime Thorpe, Yorkshire, where suitable back-up facilities are already available. This would cost approximately £2 million. Another alternative would be for the board to persuade the Americans to build their larger plant in Britain.

But in any case, Mr Grainger believes that much of the future development of fluidised bed combustion will take place in the United States because American electricity production, unlike British, is expanding. Fluidised bed combustion would thrive on an expansion of generating capacity based on a low grade fuel, and it can also greatly reduce the emission of both sulphur and nitrogen oxides in the flue gases. The keener edge to the environmentalist lobby in the United States and vast reserves of low grade coal combine to make fluidised bed combustion a more attractive proposition there.

The NCB hopes that any technical information gained by the United States in its development of fluidised bed combustion, or any of the other processes, will be re-exported back to Britain. Clearly, this sort of cooperation is the

only sensible way for industrial societies to solve their energy problems.

Family planning: a safer pill

Sally Owen

A NEW 'pill', containing an ultra-low dose of hormones, is expected to set a new standard of safety for oral contraceptives. Ovranette, which has been medically approved by the Family Planning Association, contains only one-third the amount of hormones in existing low-dose pills.

John Wyeth and Brother Ltd, manufacturers of the new pill, consider the lower hormone content to be an important step forward in oral contraceptive formulation, in line with the recommendation of the Committee on Safety of Drugs in 1969 that oral contraceptives should not contain more than 50 µg of oestrogen.

For some years now a small increase in the risk of blood clotting has been associated with the oestrogen in oral contraceptives. It is felt probable that doses lower than 50 µg a day make this increase even more minute. Following the committee's recommendation several low-dose contraceptives were brought to the market, most of them containing 50 µg of oestrogen.

Ovranette contains 30 µg oestrogen and 150 µg progestogen, which means that a monthly intake of about 11 mg oestrogen is reduced to 3.78 mg. This new pill should reduce the hazards of blood clotting not only because of its low oestrogen content but also because of the low dose and special nature of the progestogen involved.

Ovranette contains a progestogen called norgestrel which is not converted to oestrogen in the body. In fact it is actively anti-oestrogenic, reversing some of the changes brought about by oestrogen. It is thought therefore that the increase in blood clotting will be reduced even further.

One of the problems posed by low-dose oral contraceptives has been the unacceptable levels of breakthrough bleeding or spotting between normal menstrual periods. In clinical trials Ovranette compared well with other combined oral contraceptives, in that the incidence of intermenstrual bleeding was low. The cycle length among trial patients was invariably 28 days, with an almost unchanged menstrual flow. Other side effects, such as feelings of nausea, breast tenderness and depression, were less evident in trial patients who had been taking a pill containing larger amounts of hormones.

news and views

Even small meteoroids are fluffy

EVER since the enormous advances made in radar during the Second World War, scientists have been using radar techniques to study meteors. Most of the pioneer work was carried out at Jodrell Bank, but nowadays the world is dotted with radar research stations busily analysing the echoes returned from the columns of ionisation left behind by ablating meteoroids and relating these echoes to the physical parameters of the particles.

In an important article in a recent edition of the *Journal of Geophysical Research* (78, 8429; 1973), Verniani of the Istituto di Fisica dell'Atmosfera del Consiglio Nazionale delle Ricerche, Bologna, Italy analyses the physical parameters of 5,759 faint radio meteors recorded at Havana, Illinois in 1962 under the Harvard Radio Meteor Project. A system of six radar stations has been operated at Long Branch, Havana at a frequency of 40.92 MHz with a peak transmitted power of around 2 MW. Trains of pulses transmitted from the double trough antenna at the main site were reflected from the electron columns produced by meteoroids in the upper atmosphere (at heights of around 90 km) and observed at a series of five remote sites. These remote sites were connected to the main site by microwave links, the echoes being displayed on oscilloscopes and recorded on film.

The Fresnel pattern produced as the incident meteoroid moves through the antenna beam is used to determine the meteoroid velocity, and the measurement of the echo amplitude yields the electron line density (the number of electrons per unit length along the train). Each of the Fresnel patterns received at the different stations furnishes a value of velocity and line density at a particular time. Velocity can be measured to $\pm 5 \text{ km s}^{-1}$. Deceleration can be obtained from the variation of velocity with time and, by fitting a parabola to the values of line density, the maximum line density and the total number of electrons in the train can be calculated. Measurements of the zenith angle of the apparent radiant and the altitude of the echo points can be used to find the height of the reflection point from the echo range. The deduced ionisation curve gives the beginning and end height of the meteor train. The use of a few assumptions allows the computation of the original mass of the meteoroid when it was outside the Earth's atmosphere, the radio magnitude, the meteoroid density and the ablation coefficient (this coefficient relates the rate of mass loss to the deceleration; it is a function of the heat of fusion of the meteoroid material and the degree of fragmentation. Measurement of the coefficient gives a vital clue to the chemical composition and the physical structure of the meteoroid). It is this multiplicity of data about individual meteors that makes the observations taken at Havana and this work of Verniani's so important in extending knowledge and understanding of these meteoroid particles.

Verniani finds that the 5,759 radio meteors analysed (mean mass of $1.6 \times 10^{-4} \text{ g}$, and radio magnitude +8.2) have a mean geocentric velocity of 36 km s^{-1} and decelerate at the rate of 13 km s^{-2} on hitting the atmosphere. These meteoroids produce electron trains of length 11 km containing around 2×10^{16} electrons, the electron line density maximising at a value of $3 \times 10^{10} \text{ cm}^{-1}$. This maximum

ionisation occurs at a height of 92 km, the beginning height being 95 km and the end height of the train 88 km.

How do these observations tie in with other work on meteors? First, the mean density of the meteoroids is found to be 0.8 g cm^{-3} , and even more important this density is independent of mass in the range 10^{-6} to 10^{-8} g , suggesting that meteoroids have a porous, loosely conglomerate structure similar to that found for photographic and visual meteoroids having masses 10^6 times greater (see Verniani, *Space Sci. Rev.*, 10, 230; 1969). This favours a cometary origin for these particles as opposed to an origin as asteroidal collision debris, a process which would produce particles of much higher density. Verniani also finds that sporadic and shower meteors are very similar, indicating that the cometary origin of most meteoroids extends right down to 10^{-6} g .

Does the low value for the meteoroid density provide an indication of the structure of comets? The 'gravel bank' model of Lyttleton (*Comets and their Origin*; Cambridge University Press, 1953) has comets consisting of a cloud of widely spaced, small, rocky particles whereas Whipple (*Astrophys. J.*, 111, 375; 1950) postulates that comets have nuclei, these being a conglomerate of dust particles bound together by solid ices. Solar heating leads to the formation of a porous dust crust surrounding a central dirty iceball; this crust then breaks up irregularly to produce meteoroids, the low densities of the meteoroid being caused by their spongy texture, the holes in the sponge having been filled with ice when they were in the cometary nucleus. So the low density can be easily accounted for by Whipple's model; with the 'gravel bank' one would have to postulate that the gravel was itself in a low density form and this would have repercussions on the ease with which comet tails are formed. These particles, however, could be easily produced if comets are accreted from interstellar clouds by gravitational focusing. The constancy of density with mass indicates that micrometeoroids, particles of masses less than 10^{-6} g which by radiating away their heat energy float to ground and do not ablate, could be 'fluffy' and of low density whereas previously these particles were thought to have densities around 3.5 g cm^{-3} , the larger radio and visual meteoroids simply being made up of collections of these micrometeoroids.

The low density value for radio meteors is further supported by the results obtained by Verniani for the train length. The mean train length of 11 km is much shorter than was theoretically expected when meteoroids were thought to be solid, compact, stone or iron particles which simply dissipated the energy obtained from the impacts of individual air molecules by surface vapourisation of the meteoric material. The short train values lead Verniani to the conclusion that these small radio meteors often fragment, this being the same conclusion that Jacchia drew from his observations of faint photographic meteors and that Jones and Hawkes (*Nature*, in the press) draw from their work on very faint (8th magnitude) "image intensifier plus television" meteors observed at the University of Western Ontario. It seems that all meteoroids between the mass limits of 100g and 10^{-6} g are porous, fragile, crumbly objects made up of loosely conglomerate spongelike material; they all originate in comets and it is to be expected that the mean velocity of the meteoroids is independent of mass, a fact which unfortunately cannot be checked with data from radio meteors because of the height-ceiling selection effect inherent in the technique.

D. W. H.

Synthesis of ideas on early man

from a Correspondent

IN New York recently an attempt was made to bridge the gap between the palaeontologists who find and describe hominid fossils and those who seek to interpret them. A week-long conference (January 26 to February 2) was sponsored by the National Science Foundation to deal specifically with African Plio-Pleistocene hominids, the aim being to consider the fossil evidence, identify the outstanding problems and to formulate research strategies.

The first part consisted of public presentations of the evidence by the discoverers of the fossils. W. W. Bishop (Bedford College, London) began by presenting the chronological and faunal framework for the East African sites. The physical data on chronology, both isotopic and palaeomagnetic, combine with faunal correlations to provide a formidable backcloth for the hominid remains. He emphasised the primary, but not always infallible role of physical dating methods; it was the incompatibility of the fauna with the early dates from Kanapoi in Kenya that led to an eventual revision of the isotopic dating evidence. The situation in East Africa is in sharp contrast to that in South Africa where, although faunal correlation data are being accumulated,

there is a total lack of physical data on chronology.

There was unanimous scepticism about the use of geomorphological evidence to date the South African cave sites. It was felt that uncertainties about the dates of inception of planation cycles, the conflicting evidence from deep-sea cores, documentation of highly variable erosion rates and the influence of various lithologies on nick-point progression make such estimates, based on geomorphology, unacceptable as primary evidence. Hopes for adequate dating of these sites currently rest on fission track and palaeomagnetic studies. Bishop also presented the hominid remains from the Baringo basin (Kenya) and B. Patterson (Harvard University) wittily explained the context of the Kanapoi and Lothagam sites, and in doing so showed a healthy reluctance to elevate his hominid fossils to a position above that of the other fauna. He told of how the Kanapoi site was found as the result of a 'Sunday excursion' for which his field expeditions are apparently notorious.

M. D. Leakey (Olduvai Gorge) showed, albeit unintentionally, what a profound influence the painstaking archaeological, palaeontological and geological studies at Olduvai have had on the standard of field and laboratory work at other sites. F. C. Howell (University of California, Berkeley) and R. E. F. Leakey (National Museums

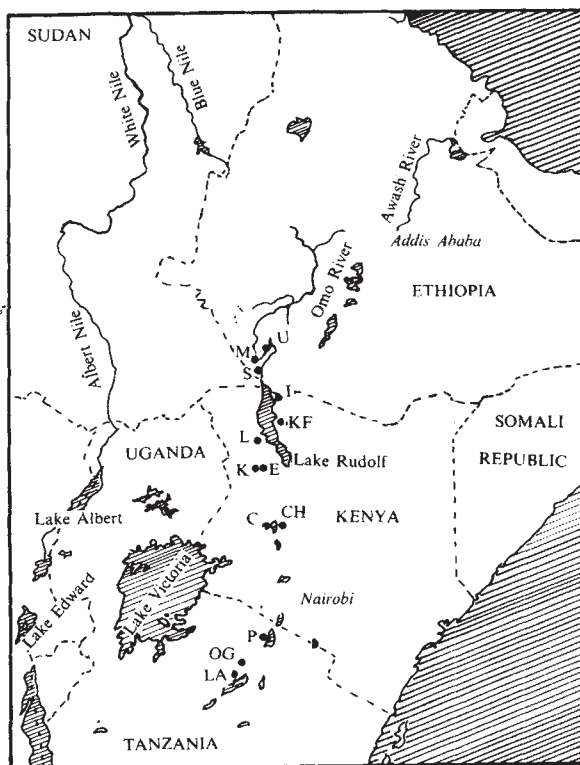
of Kenya) showed how successful this sound methodology can be when applied to the extensive fossiliferous exposures in the lower Omo Valley and in the area east of Lake Rudolf. There is apparently at Omo, as at Olduvai, evidence for an environmental change about midway in the sequence and this may be an explanation for some of the inconsistencies that exist in the isotope dates and the faunal evidence between Omo and East Rudolf.

The evidence from the South African cave sites was reviewed by C. K. Brain (Transvaal Museum) in a presentation that was perhaps the highlight of the meeting. Recent excavations at Swartkrans have revealed a new stratum, the orange breccia, and evidence of solution channels in the outer cave breccia. It is now known that SK15, one of the '*Telanthropus*' mandibles comes from this channel fill and is probably not contemporaneous with the other hominid fossils. The possibility that other hominids were associated with similar channels may vitiate the importance of the South African hominids as being a sample from a limited time range and thus being better indicators of early hominid variability than the more temporally separated East African sample. Brain's elegant, and beautifully illustrated experiments using captive cheetahs and the companion investigations of leopard lairs may explain the curious differences in skeletal preservation between bovids and primates at the cave sites.

The recently prospected fossiliferous exposures in the Awash Valley in north-east Ethiopia were described by D. Johanson (Case Western Reserve University). Faunal correlations indicate an age of about 3 million years for the exposures at Hadar which have yielded hominid leg bones and a cranial fragment. Fragmentary though some of these specimens are, the excellent preservation of other vertebrate fauna suggests that these will not be the last hominids found at Hadar.

The remaining four days were held in the comfort of the Wenner-Gren Foundation. Sessions were devoted to morphology, demographic aspects, cultural evidence, patterns of diversity and implications of taphonomic evidence for behaviour patterns. Of the current research problems differences in methodology were the most evident. Views polarised into those who considered that it was necessary to establish patterns of morphological variability before phylogenetic and functional interpretations of the hominids could proceed. Others considered that examination of the material should be preceded by hypothesis formation about broader patterns of hominid behaviour and variability: suffice it to say that this

Map of some of the Plio-Pleistocene hominid localities in eastern Africa. M(Mursi), U(Usno), S(Shungura) Formations, lower Omo basin; I, Ileret sector; KF, Koobi Fora sector of East Rudolf area; L, Lothagam; K/E, Kanapoi and Ekora of lower Kerio drainage; CH, Chesowanja, Baringo basin; K, Kanam; P, Peninj, Natron basin; OG, Olduvai Gorge.



matter was not resolved! Opinions differed about the role of multivariate statistics, the criteria for establishing population diversity, the association of culture with any particular hominid group, the interpretation of bone 'tools' and the significance of differences in endocast morphology; in fact few 'stones' were left unturned.

Despite differences in approach, perhaps an uncomfortable level of broad agreement was reached about the existence of cranial, dental and postcranial morphotypes (though one suspects that for some participants there were too many cranial morphotypes to fit the teeth or postcranial material!). Future research problems that were identified include more comparative work on endocranial morphology, dental wear patterns and postcranial variability and further research into the implications of dental eruption patterns of hominines and current taphonomic studies for hominid behaviour.

This conference successfully brought together workers whose research approaches are different, but whose common aims are to discover more about the structure and behaviour of early man. This meeting complemented the workshop conference held in Nairobi in September 1973, which attempted to synthesise the results of work at Omo and East Rudolf. The publications resulting from these two meetings will attest to the growing scientific maturity of hominid studies and show the success of the international and multidisciplinary approach to hominid palaeontology which has been a feature of the research both at Omo and at East Rudolf.

Are accretion disks stable?

from a Correspondent

THE stability of accretion disks in binary X-ray sources comes under critical review in an article by Lightman and Eardley in a recent issue of *Astrophysical Journal* (187, L1; 1974). The accretion of material by a compact object (a neutron star or a black hole) from its companion in a binary star system is thought to be the main source of power for the luminous X-ray sources that have been discovered within our galaxy during the past few years. Because of the rotation of the binary system, transferred material is forced by the conservation of angular momentum initially to form a disk around the compact object. Differential rotation and viscosity, in the disk, transfer angular momentum outwards, allowing the matter to spiral inwards, liberating its gravitational potential energy as it does so. If the compact object is a neutron

star, most of the energy is liberated at the surface of the star itself, where the accreting material crashes into it. But, if the compact object is a black hole, then all the luminosity comes from the disk, and the matter arriving at the inner edge of the disk is swallowed quietly by the black hole. Thus for accretion by a black hole, widely speculated to be the case for the X-ray source Cygnus X-1, the stability of the accretion disk is an all important matter.

The most uncertain element in all models of accretion disks is the viscosity—ordinary molecular viscosity is far too small, and authors usually invoke turbulence or magnetic fields. A common (*ad hoc*) assumption is that the effective kinematic viscosity (with dimensions of length²/time) is roughly equal to the vertical thickness of the disk (the supposed size of a typical turbulent eddy) times some constant fraction of the sound speed (since supersonic turbulence would be rapidly dissipated). The difficulty arises in the inner regions of the disk where radiation pressure, rather than ordinary gas pressure, dominates the vertical structure. In this region, it is unclear what the 'sound speed' relevant to turbulent motion should be, and it is usually assumed to be that dictated by the radiation. Lightman and Eardley, however, have demonstrated that, in this part of the disk, this assumption about the viscosity is not consistent with the disk being in a steady configuration. They show, moreover, that if the disk is forced initially to conform to the previously calculated 'steady' configuration, it evolves secularly, breaking up into rings. The reality of these rings is, however, questionable, since their formation depends on being able to start in a supposed 'steady' situation, which has already been shown not to be self-consistent.

The reason for this 'instability' is fairly simple. The assumptions mentioned earlier lead to the conclusion that in this part of the disk, the viscous stress is inversely proportional to the surface density of the disk. Thus, if a small region of the disk has a lower than average surface density, the viscous stress, and hence the radial velocity, there will be higher than average, and so the surface density will decrease still further.

The authors suggest two possible alternatives. First, if one assumes rather that the 'sound speed' relevant to turbulent viscosity is that given by the gas pressure, then the resultant steady accretion is self-consistent. Second, if the assumptions made are roughly correct, then the idea of a steady accretion disk must be abandoned. The observed variability of the source Cygnus X-1 on timescales as short as milliseconds might lead credence to this idea. In any

event this contribution serves as a timely reminder to theoretical astronomers that '*ad hoc*' assumptions which seem to work should not have too great a trust put in them.

Darwin glass related to tektite fall?

from our Geomagnetism Correspondent

THE material that has come to be known as Darwin glass was first recognised and described just 60 years ago by Suess who found large quantities of it along a north-south track some 3 km to the east of Mount Darwin in Tasmania. About 50 years later, geochemical studies and the discovery that the glass contains coesite led to the view that the Darwin glass must have been formed by impact—a conclusion which was strikingly confirmed two years ago when Ford (*Earth Planet. Sci. Lett.*, **16**, 228; 1972) discovered Darwin Crater, a circular depression with a diameter of about 1 km lying some 4 km or so to the east of the most southerly of Suess's deposits.

It is now known that there are far more examples of glass in the vicinity of Mount Darwin than Suess had realised; and with the aim of confirming the genetic relationship between the original occurrences and the more recently discovered crater glasses, Gentner *et al.* (*Earth Planet. Sci. Lett.*, **20**, 204; 1973) have used both potassium-argon and fission track methods to date glass samples from the interior and rim of the crater and from areas up to 2 km away from the rim. The mean potassium-argon and fission track ages are 0.70 ± 0.08 and 0.74 ± 0.04 million years, respectively, giving a combined age of 0.73 ± 0.04 Myr. This compares with the fission track age of 0.72 ± 0.02 Myr for the longer-known glasses, obtained previously by Gentner *et al.* (*Geochim. cosmochim. Acta*, **33**, 1075; 1969).

Gentner and his colleagues thus conclude that all the known Darwin glasses were produced at the time of the impact that formed Darwin Crater 0.73 Myr ago. But there may be more in this than simply a study of the relations between glass deposits. That the Darwin glasses occur geographically close to the Australasian tektites has been obvious for some time; and what Gentner *et al.* have now done is to show that the glasses and the tektites have the same age. This is apparently the first evidence obtained that an impact crater was formed at the time of the Australasian tektites. It seems hardly likely that this is a coincidence; and yet it is unlikely that an impact forming a crater as small as the Darwin Crater could be

responsible for the large Australasian tektite field. One reconciling conclusion is that the meteorite or comet fall (if such there was) leading to the formation of the Australasian tektites produced a number of craters, of which the Darwin Crater is but one. If so, a careful search in the area should reveal them in time.

No role yet for poly (A)

from our
Molecular Genetics Correspondent

ONE of the most intriguing questions in the area of messenger RNA production in eukaryotic cells is the role of the sequence of polyadenylic acid (poly (A)) which almost all the messengers seem to carry at their 3' end. A sequence of about 200 residues of adenylic acid is added, probably one base at a time, to the 3' end of the apparent mRNA precursor, heterogeneous nuclear RNA (Hn RNA), after or during its synthesis from chromatin. After cleavage of a 3' terminal sequence containing the poly (A), transport to the cytoplasm is succeeded by association with ribosomes for translation.

One speculation about the possible role of poly (A) is refuted by the results reported by Bard, Efron, Marcus and Perry in the February issue of *Cell* (1, 103; 1974). The idea that the poly (A) might be necessary solely for nucleocytoplasmic transport has previously been rendered less likely by observations that it is found in tumour viruses whose expression is confined solely to the cytoplasm and also in mitochondrial messengers where no transport is implicated. One inference which might be drawn from these results is that the poly (A) is implicated in some role in translation, perhaps controlling the stability of mRNA or its capacity to act as template for the ribosomes. Although the sequence of poly (A) on a messenger seems to shorten progressively with time passed in the cytoplasm, studies of mRNA turnover showed that this is not correlated with any increase with time in the probability of mRNA degradation.

The possible role of poly (A) in controlling translational capacity has therefore been examined by Bard *et al.*, using two types of experiment: in the first, they examined the abilities of messengers bearing different lengths of poly (A) to be translated within the mouse L cell; and in the second they compared the activities as templates *in vitro* of mRNAs either possessing or stripped of their poly (A) sequences.

Old and new mRNAs can be preferentially labelled in the L cell cytoplasm by labelling with $^{32}\text{PO}_4$ for 20 h but with ^3H -adenosine for only 1.75 h. When

polysomes are then extracted and analysed, the ^{32}P label identifies older messengers whereas the ^3H label is found in newly synthesised messengers. The new messengers possess a length of poly (A) of about 180 residues, but in the old messengers its length is much reduced and may be as small as 50 bases. Bard *et al.* found that there is no difference in the ratio of new to old mRNA in various polysomes fractions, implying that both classes of mRNA share the same translational capacity.

An alternative possibility, that overall translational efficiencies are different but that the same ratio of initiation to elongation is maintained, was excluded by experiments utilising temperature shock. This treatment disaggregates polysomes by preventing initiation of protein synthesis; ribosomes therefore run off their current messengers to release free messenger ribonucleoprotein. Lowering the temperature allows the polysomes to reform by utilising the released mRNP. After labelling old mRNA by incubating cells for 2 h with ^3H -uridine followed by a 21 h chase, and labelling new mRNA by incubation for 1 h with ^{14}C -uridine, Bard *et al.* found that reassembled polysomes display a ratio of new:old mRNA very close to that of the control. The capacity to initiate protein synthesis of both classes of messenger must therefore be virtually identical.

Because these experiments compare messengers possessing different lengths of poly (A), Bard *et al.* then turned to *in vitro* experiments to compare messengers with or completely without poly (A). By using a 3'-OH exonuclease to degrade the poly (A) sequence, and by then utilising only messengers which had retained their original size (to exclude those suffering other breaks), they isolated a messenger fraction identical to that usually extracted from L cells, but lacking poly (A). No difference could be found in the translational capacity of the deadenylated mRNA compared with the messengers possessing poly (A), using an *in vitro* system for synthesising proteins derived from wheat embryo.

Poly (A) therefore seems to lack any role in controlling the activity of messenger RNA in protein synthesis either *in vitro* or *in vivo*. The ubiquity of poly (A) in eukaryotic messengers (the only well established fraction lacking it is that coding for the histones) implies that it must have some important function. Previous experiments have excluded control of messenger lifetimes as its function, leaving it with no role apparent at the level of translation. Although preventing the addition of poly (A) halts the production of mRNA from HnRNA, the presence of poly (A) in messengers not derived from the nucleus suggests

that it must have some cytoplasmic function. No reconciliation of this paradox is evident at present: poly (A) remains a sequence in search of a function.

Inside the sickled red cell

from a Correspondent

CURRENT interest in sickle cell anaemia itself and in the behaviour of deoxy sickle cell haemoglobin was the subject of recent comment in these pages (see *Nature new Biol.*, 242, 33; 1973). This interest has been stimulated, partly by increased support in the United States for work on the clinical management of patients with sickle cell anaemia and tests of possible therapy, and partly by new insights into the aggregation of deoxy Hb-S from a variety of techniques, in particular electron microscopy and fibre type X-ray diffraction studies. There is now a considerable literature on the aggregation of deoxy Hb-S into an ordered phase, starting with the birefringence studies of Sherman in 1940, even before the recognition of Hb-S as a haemoglobin variant.

One of the latest additions to this literature is an optical study by Hofrichter, Hendrickson and Eaton (*Proc. natn. Acad. Sci. U.S.A.*, 70, 3604; 1973) on the orientation of deoxy Hb-S molecules within the long straight fibres which have been shown by electron microscopy to make up the ordered phase. The fibre model proposed by Finch and colleagues (*ibid.*, 70, 718; 1973) is a microtubule composed of stacked rings of six Hb-S molecules. Since each ring is rotated slightly, relative to the preceding ring, the structure can alternatively be envisaged as six intertwined helical filaments, each having about 48 Hb-S molecules per turn. Neither electron microscopy nor X-ray diffraction has as yet provided definite information on the orientation of the molecules within the fibre, or the role of the mutation sites (Glu A3(6) $\beta \rightarrow$ Val) in the intermolecular contacts.

Eaton and his colleagues have made polarised absorption measurements on single sickled red cells and single crystals of deoxy Hb-S. The polarisation ratio of sickled cells gives a lower limit for that of an individual fibre, and taken in conjunction with the absorption properties of the haemoglobin molecule restricts the direction of the long molecular axis (x) to within 22° of the fibre axis. The highly anisotropic absorption ellipsoid of the haemoglobin molecule required for this work was calculated for the Soret band, since optical studies on haem protein single crystals by Eaton and colleagues had shown this haem chromophore transition to be nearly perfectly in-plane. The haemoglobin ellipsoid was

obtained by summing the squared projections of all the haem planes on to the form ellipsoid of the molecule, and this was then transformed into the crystallographic system.

After defining the limits of the orientation of the x axis, and using the Finch stacked-ring model for the molecular positions, with the requirement that at least one mutation ($\beta 6$) site is part of an intermolecular contact, the measured polarisation ratio for the Soret band requires that the true molecular dyad (y)

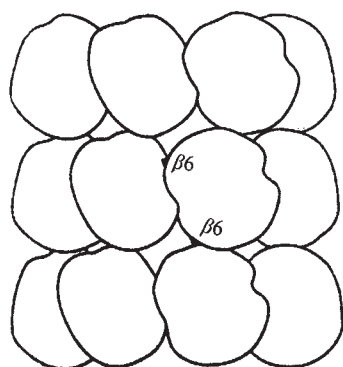


Diagram of one of the three classes of model for the orientation of molecules in the sickle cell fibre allowed by the optical results of Hofrichter *et al.* and by the additional constraint that at least one $\beta 6$ site is part of an intermolecular contact. If the maximum possible tilt of the x axis is used, the other $\beta 6$ residue of a molecule is in a position to make an interring contact.

axis passes through some part of an adjacent molecule in the same ring of six molecules. This range of orientation for the y axis turns out to be approximately perpendicular to those proposed in previous models.

The new information obtained from the optical studies allows three classes of model to be considered, which differ in respect of the possible dispositions of the $\beta 6$ sites. In all three models a $\beta 6$ residue could assist in stabilising intraring contacts. If the maximum possible tilt of the x axis is used, the other $\beta 6$ residue of a molecule is in a position to make an interring contact, though more extensive polarised absorption measurements will be required to establish this interesting possibility. In any case the absorption polarisation work, which is technically very impressive, has already provided new insights into the detailed structure of the deoxy Hb-S fibre and may suggest new approaches to the practical problem of inhibiting the aggregation process.

Not all at sea on the Channel

ON Thursday, January 31, a large contingent of French scientists converged

upon London to meet their English counterparts for two days of discussion on the geology of the English Channel. Although physical constraints required that the scientists meet in either London or Paris, metaphorically they met half way—a great deal of academic *entente* was evident throughout the proceedings. The meeting, jointly organised by the Royal Society and the Marine Studies Group of the Geological Society, was a great success. And it was not without its surprises: “I began”, said one of the organisers, “by asking for volcanoes, and in the end I was given ice”.

But despite the more surprising aspects, the meeting showed that a new understanding of Channel geology has emerged which will not only have important implications in future studies of the geology of north-western Europe, but which may well result in a considerable reappraisal of established theories concerning that area. This is largely due to the amount of detail about the basement geology which has been collected by the French scientists.

There is evidence to indicate that the region has had an important tectonic history, the effects of which have not been fully appreciated in the past. There have been two major phases in the history of the Channel, probably interspersed with many minor tectonic events. A. J. Smith (University College, London) believes that both phases are intimately associated with events in the history of the spreading Atlantic Ocean. Soon after the end of the Hercynian orogeny about 280 million years ago, the Atlantic began to open along a trend aligned with that of the Channel, and many of the primary structures of the Channel originated at this time. During the Early Cretaceous, probably about 130 m.y. ago, the second phase developed as the Bay of Biscay opened and the axial trend of the Atlantic changed to its present orientation.

Consideration of the present structure and geology of the Channel shows that its tectonic history must have had some influence on the geological evolution of northern France and southern Britain. The Channel can be divided into three areas on a structural basis: an area to the west of the Anglo-Norman Ridge; a central area between this ridge and the Bembridge-St Valery Line; and a third area to the east. It is as a result of studies of the central area that geologists have come to appreciate the importance of the Channel's tectonic history. Within this central region two east-west structures downturn beds to the south. Geophysical data indicate that the tectonic activity in the Channel has been largely restricted to vertical block movements of the basement (which to some extent are still continu-

ing), and the surface structures of the central area are probably reflections of the basement structure. But, the important fact is that the southernmost of these two structures joins the Bembridge-St Valery Line and becomes deflected onto the French coast. The deflected structure has become a very minor feature by the time it reaches the coast, but, significantly, it is in perfect alignment with major structural features which pass through northern France, and into the Massif Central. Furthermore, the northern extension of the Bembridge-St Valery Line passes into important structures in south Dorset.

The implication is that major structural features which have previously been interpreted without any consideration of the role of the events which are manifest in the Channel, will now have to be examined more closely. Are the Mendips really just part of the Hercynian Front, or do they in fact reflect the vertical movements of the basement rocks of this area? And was the Weald really folded into its present structure by epeirogenic movements during the Alpine orogeny; or is D. Curry (University College, London) correct in the reiteration of his old idea that the Hampshire, London and Somme basins did not form separately?

But despite the implications raised by these issues it was largely the well-received theory of G. A. Kellaway (Institute of Geological Sciences, London) and his colleagues which dominated the discussions. Early on in the meeting Smith had suggested that there may have been considerable volcanicity accompanying the first major tectonic phase, and that the lavas which accumulated may be responsible for the present isostatic balance which leaves the western area of the Channel as a basinal depression. Unfortunately none of the other speakers could provide proof for this attractive hypothesis, and so Kellaway, unable to provide a volcano, provided a glacier instead. He has managed to find an impressive amount of evidence, including boulder clay from the Slinden Pipes, to show that during the Anglian glacial advance the Channel was filled with glacial ice which moved eastwards from the edge of the continental shelf, and deposited the abundant erratics which today litter the coastline of southern England.

Kellaway also believes that the ‘raised beaches’ around the Sussex coastline are not raised beaches at all, but are marginal outwashed deposits. His theory could certainly provide an acceptable explanation for the previously mysterious buried channels and valleys and for the Hurd Deep, all of which may be the result of subglacial erosion.

Accuracy of tree-ring dating

from our Archaeology Correspondent

THE correction of the radiocarbon time scale by means of tree-ring dating has profoundly affected archaeological chronologies, and has important geophysical implications. Hitherto, the calibration has been based upon a single 7,400-year tree-ring sequence, established for the bristlecone pine by Ferguson. The production of an independent tree-ring sequence for the same species, extending back to 3535 bc (LaMarche and Harlan, *J. geophys. Res.*, **78**, 8849; 1973) is thus of considerable importance. That it should differ by only two years from the original Ferguson sequence offers strong corroboration to both.

In both cases the trees examined are in the southern White Mountains of California. In the new study wood from a limited area on Campito Mountain, at elevations over 3380 m, was used; Ferguson's earlier study (page 237 in *Radiocarbon Variations and Absolute Chronology*, edit. by Olsson; Almquist and Wiksell, Stockholm, 1970) was based on wood from a lower elevation at Methuselah Walk, about 10 miles south of Campito Mountain. The Campito sequence is based on samples from 37 living trees, which take it back to ad 600, and woods from 70 dead trees were utilised to carry the sequence back to 3535 bc. There are three potential error sources in such a count. To the risk of extra intrannual rings is added that of locally absent rings. And, third,

there is the possibility of the mismatching of rings from one specimen to the next as the record is built up. LaMarche and Harlan conclude that false latewood and 'earlywood' bands can be recognised by their cell structure, so that the first problem is not difficult. They offer arguments to show that when there is a possibility of a few annual rings being uncoupled in the chronology (that is of missing rings), "the error in the chronology from this source should only be of the order of one year". Cross-dating errors can be controlled by the recognition of characteristic frost damage rings, often shared by the different trees in a specific area in very cold years.

The importance of the work is that these considerations were put to the test by a detailed comparison of the Campito Mountain chronology with the Methuselah chronology. The cross dating is demonstrated objectively by means of cross-correlation analysis. This was first carried out using the dates originally assigned to the Campito chronology, and the coefficient obtained had negative or low values for part of the period. When the Campito dates were adjusted by insertion of two missing rings, the cross-correlation coefficients for the earlier period were positive and highly significant. The authors conclude: "The incidence of false rings that might erroneously increase the age is so small as to preclude explicit evaluation. The only important potential source of error seems to be locally absent rings. On the basis of the close agreement of the estimated number of rings absent from

the Campito chronology with the observed discrepancy between the Campito and Methuselah chronologies, it is highly probable that the absolute error of the Methuselah chronology at 3435 bc is, in fact, zero".

This conclusion is of fundamental interest to archaeologists and others who seek to use the dendrochronological calibration of radiocarbon, because hitherto this has been based principally on the Methuselah sequence. Although the accuracy of this sequence has not seriously been questioned, independent corroboration was desirable. Sceptics could yet argue that sequences obtained from trees of the same species, from locations only 10 miles apart, are not necessarily independent, but the results must strengthen the bristlecone pine sequence.

They cannot help, however, with two other problems of the calibration. In the first place there is the possibility that as a result of the diffusion of recent tree sap across older tree rings, the radiocarbon determination obtained from a given ring may yield a date substantially later than that of the ring's formation (Berger, *Phil. Trans. R. Soc. A*, **269**, 33; 1970). And, second, the atmospheric concentration of radiocarbon in southern California may not always have been the same as, for instance, in Europe. These two problems can only be resolved by tests outside California. The long tree-ring sequence (mainly of oak) at present being established in Belfast may ultimately answer both questions. Meanwhile, comparison of radiocarbon dates obtained from dendrochronologically-dated bristlecone pine samples with radiocarbon dates obtained from archaeologically and historically dated samples from ancient Egypt at least produces no conflict. But the errors associated both with the radiocarbon determinations and with the Egyptian archaeological contexts are so large that this comparison is far from conclusive.

A problem related to these is that of the shape of the curve which relates calendar and radiocarbon dates. The version originally prepared by Suess had many kinks (page 303 in *Radiocarbon Variations and Absolute Chronology*). Other laboratories, notably at the University Museum in Philadelphia and the University of Arizona, have used smoothing procedures to give a more convenient curve, although as Burleigh has pointed out (*Antiquity*, **47**, 309; 1973) there is at present no sound reason for eliminating the kinks.

This question of the precise shape of the curve is, however, secondary: the primary problem is the general and worldwide validity of the data upon which it is based. The new Campito sequence serves to confirm one of its



Standing snags of bristlecone pine on Sheep Mountain in the Campito Mountains of California. These trees have been

dead for more than 300 years. (Photo: Laboratory of Tree-ring Research, University of Arizona.)

sustaining arguments, and is thus a first step towards the validation of the calibration as a whole.

Pion capture reveals alpha clusters

from our Nuclear Theory Correspondent

WHEN a negative pion comes to rest it is captured by a nucleus and gives it all its rest energy. Since energy and momentum must be conserved in the capture process the pion cannot be captured by a single nucleon but must interact with several nucleons at once, so a detailed study of the particles emitted provides information on nucleon correlations in the nucleus.

One of the most likely forms of nucleon correlation is their tendency, especially in light nuclei, to form α clusters, and a recent experiment by Lewis, Ullrich, Engelhardt and Boschitz (*Phys. Lett.*, **47B**, 339; 1973) on the capture of negative pions by ^{16}O provides new evidence of the presence of these clusters in nuclei.

They looked at the γ -ray spectrum emitted by a ^{16}O target irradiated with slow pions and found a strong peak due to the 4.44 MeV γ -ray from the decay of the first 2^+ excited state of ^{12}C . It is thus natural to suppose that the pion capture has broken the ^{16}O into an α particle and an excited ^{12}C nucleus, and indeed it is known that this mode of breaking up is about six times more likely than the corresponding process leaving ^{12}C in its ground state.

Since the α particle has a momentum distribution relative to the ^{12}C in the original oxygen nucleus, the recoiling carbon nuclei have the same distribution, superposed on the recoil momentum determined by the rest mass of the pion. This momentum distribution produces a Doppler broadening of the shape of the γ -ray line, and measurement of this broadening gives the momentum distribution of the α particles.

This momentum distribution can also be calculated from a simple harmonic oscillator model, thus providing a check on the interpretation of the data. In such a model, the momentum distribution of a particle moving with angular momentum L with respect to the centre-of-mass of the harmonic oscillator potential has the form $P(K) \propto K^{2L} \exp(-K^2/2Q_L^2)$, and fits to the data given $Q_2 = 75 \pm 5$ MeV/c. This may be compared with analyses of the $^{16}\text{O}(p, \alpha)$ knockout reaction that give $Q_0 \approx 75$ MeV/c. The values of Q_0 and Q_2 are expected to be similar, so this is an indication of the consistency of the two analyses.

This experiment essentially gives the distribution of the sum of the momenta

of the four nucleons emitted when pions are captured by ^{16}O . These four nucleons are most probably combined to form an α particle because the alternative interpretation of absorption on a deuteron followed by evaporation of two nucleons would give the characteristic γ -rays from the decay of ^{12}B , and these are not observed. Other possible origins of the observed γ -rays were excluded by repeating the experiment with different targets.

It is thus very reasonable to interpret these observations by the α particle absorption model and this gives further support to the idea that α clusters are present in nuclei. Similar results have been obtained for other nuclei besides oxygen.

Population energetics of kangaroo rats

from our Animal Ecology Correspondent

It has been suggested that because terrestrial herbivores have low efficiencies of consumption of net primary production (NPP), their populations generally are not limited by food (Slobodkin *et al.*, *Am. Nat.*, **101**, 109; 1966; Wiegert and Owen, *J. theor. Biol.*, **30**, 69; 1971). Most species of small mammals from the temperate biomes consume between 0.1% and 1.0% of the NPP annually. When this amount is expressed in terms of available productivity (AP)—that is, ignoring inedible parts of the plant—the values range from 0.1% to 4.0%, with a few exceptions (Chew and Chew, *Ecol. Monogr.*, **40**, 1; 1970; Wiegert and Evans, in *Secondary Productivity in Terrestrial Ecosystems* (edit. by K. Petrusewicz) 499, *Polish Academy of Science*, Warsaw, 1967; Grodzinski *et al.*, *Acta Theriol.*, **11**, 419; 1966). Sohlt has now published some interesting data on the impact of a population of the kangaroo rat (*Dipodomys merriami*) on the Mojave desert and shows that the rats take 6.9% of the NPP and 10.7% of the AP annually (*Ecol. Monogr.*, **43**, 357; 1973). The study offers some thoughts on why population densities of desert rodents are as high as they are.

Sohlt estimated population density by recapture trapping and the Lincoln Index. During the year of study the density averaged 16.2 rats ha^{-1} , a high figure for a species living in conditions where the NPP is just 1,400 megacal $\text{ha}^{-1} \text{yr}^{-1}$. (In temperate zones the NPP is between 20,000 and 30,000 megacal $\text{ha}^{-1} \text{yr}^{-1}$.) Sohlt measured resting energy expenditure, the energy cost of activity, the energy expense of growth, assimilation efficiency and calculated the total energy flow (E) through the population to be 85.5 megacal $\text{ha}^{-1} \text{yr}^{-1}$.

From his studies on growth and reproduction he deduced that the secondary productivity (P) of the population was 0.7 megacal $\text{ha}^{-1} \text{yr}^{-1}$, or 0.8% of the total energy flow. This is somewhat lower than the P/E ratio for other rodents which fluctuates between 1.2 and 3.0%. This suggests that kangaroo rats are not adapted for utilisation of as high a proportion of their total energy flow for secondary productivity as are non-desert species. Perhaps this adaptation is prudential in an environment where the amount of food production is unpredictable.

Although several species of plants were available to the rodents, they showed a strong preference for the annual storks-bill *Erodium cicutarium*. Seeds formed the major item in the diet, but leaves were consumed during the winter. Laboratory studies showed the assimilation efficiency (AE) to be 0.87. Assuming this, the amount of food that had to be consumed such that E/AE equaled 85.5 megacal $\text{ha}^{-1} \text{yr}^{-1}$ was calculated to be 97.8 megacal $\text{ha}^{-1} \text{yr}^{-1}$. A little more than 1.0 megacal was supplied by arthropods; most of the rest by *Erodium*.

Further field studies revealed that, in order to obtain this caloric input chiefly from one species of plant, the rats consumed, incredibly, more than 95% of the *Erodium* seed production and more than 90% of the total *Erodium* production. The population thus exerted a massive effect on the energy turnover of this one species, although at 6.9% of the total NPP it had little effect on the vegetation as a whole. Its impact on the survival chances of this species population would be immense if the remaining 5% of the seeds are insufficient to support as great a plant population in the succeeding year. It is a pity that the study was not continued to ascertain what were the population levels of rat and storks-bill during the following year. Populations of desert annuals are limited by rainfall so that desert rodents are unlikely to exert an important controlling effect. If rainfall was not limiting, the rodents would likely consume *Erodium* until it became scarce to find and in that way actually limit its population.

Sohlt's study shows that this population of *Dipodomys* lived dangerously on the brink of overexploiting its food resources. Wiegert and Owen suggested that low consumption of plants avoided long term oscillations of the plant populations. In deserts, where extremes of environmental factors make oscillations unavoidable, the evolution of such a damper would prove ineffective. Thus there is no disadvantage to a population of rodents consuming more than 95% of its major food source.

Glassy state good for lasers?

from our Solid State Physics Correspondent

LASER beams are often of such high intensity that the electric component of the electromagnetic wave reaches the level of the breakdown field for the medium through which it is travelling. In solids, local fractures and other damage are seen at a level of about 10^8 V m⁻¹ and in gases, plasma discharges are formed. The instantaneous field at which this takes place in solids is called the intrinsic optical damage field (the word 'intrinsic' is necessary because in solids there are other damage phenomena which are not intrinsic to the material and which are apparently caused by increased energy deposition in small imperfections in the transparent materials; these effects may occur at much lower light intensities than the electrical breakdown).

The intrinsic breakdown is interesting both from the fundamental and from the practical point of view. Electron avalanching is almost certainly the mechanism by which damage occurs and the processes by which hot electrons impart energy to a solid lattice is of great interest at present. Also, in the bid to make more and more powerful lasers, materials with the highest possible tolerance to the extremely high frequency electric fields present in the light pulse have to be found or fashioned; of special interest, of course, are the optically transparent glasses, mainly amorphous compounds with a wide band gap such as that possessed by many oxides. Here, the question of electron transport in a disordered lattice presents a double problem; first, because the theory of high field transport in any material with a wide band gap is not yet well developed and, second, because the theory of carrier scattering in disordered systems is more complex than that in crystalline lattices and is also in an embryonic state; some courageous attempts at predicting breakdown from data on lattice vibrational modes in an amorphous dielectric have, however, been made, with fair success (Lynch, *J. appl. Phys.*, **43**, 3274; 1972).

Thus the need for utilitarian data on the behaviour of glass optical elements in laser systems leads into a distinctly challenging area of research and leaves one to sink or swim, and perhaps to grasp at straws of data as they come past. Such a straw, perhaps, is a first report of a comparison of intrinsic damage fields in some glasses, some polycrystalline solids and some comparable single-crystal materials by Fradin and Bass, of Raytheon Research and the University of Southern California, re-

spectively (*Appl. Phys. Lett.*, **23**, 604; 1973).

The comparisons are between quartz and fused silica, polycrystalline and single-crystal potassium chloride and a germanate glass and its crystalline form. First, the fused silica did not show breakdown until a light intensity of 10^8 GW cm⁻² was reached, five times that at which crystalline quartz broke down. This factor of five in intensity corresponds to a factor of 2.2 in the relative damage fields. By contrast, polycrystalline potassium chloride broke down at about the same intensity as its single-crystal form (about 8 GW cm⁻²). Thus, both types of silica seem to be very good laser materials but, what is more, a large improvement seems to be gained by virtue of the microscopic disorder of the amorphous form. The disorder in this material is on the scale of a few lattice constants, taking the form of a dispersion of bond angles in an otherwise continuous and compositionally ordered network. The polycrystalline potassium chloride, of course, is disordered but is made up of crystallites which are many hundreds of lattice constants in size. Thus, grain boundaries do not seem to interfere with the progress of the gross damage effects (fractures and so on), which are the indicators of when the

intrinsic damage fields are reached, but microscopic disorder apparently damps the breakdown effect quite drastically.

Although this is only a straw in the wind, a set of data for only one amorphous/crystalline pair, it is tempting to grasp at it and say, as the authors do, that the disordered network is more efficient at extracting energy from the electrons before they reach the energy necessary for impact ionisation: that is, it is more difficult to heat the electron distribution in the disordered material. This is, however, equivalent to saying that the electron mobility in amorphous silica at high fields is considerably lower than that for the crystalline form; this is unlikely, since Hughes has found that, for fields up to 10^7 V m⁻¹ (though impurity content caused some variation) the electron mobility and lifetime in synthetic fused silica was generally higher than for pure crystalline quartz. (See, for example, *Nature*, **246**, 190; 1973). Nevertheless, the study of high field breakdown in solids induced by a laser, when carried further, may well prove quite a versatile and useful method for investigating the transfer of energy from hot electrons to their solid host medium, with unique possibilities for the localisation and shortening of duration of the accelerating fields.

Singing muscles in a katydid

from our
Insect Physiology Correspondent

INSECTS are commonly regarded as cold blooded, or poikilothermic—their temperature agreeing with that of the environment. But it was well known to Newport, writing in Todd's *Cyclopaedia* in 1838, that the honeybee without visibly moving increases its body temperature well above that of its surroundings; and it is now known that this change is accompanied by action potentials in the thoracic muscles without vibration. It has been known since Girard in the middle of the last century that hawk moths increase their thoracic temperature by as much as 10° C by vibrating their wings. Such a rise in temperature increases the efficiency and frequency of wing muscle contraction. Recourse was had to this effect in trying to explain the very high rates of contraction and relaxation in the flight muscles of insects, which far exceed those of vertebrates. The rate of wing beat in the humming bird is 30–50 cycles per second: the blowfly will beat its wings at 150 cycles per second, a rate too rapid to be explained by a rise in temperature alone.

It was suggested that these wing muscles were operating in a state of incomplete tetanus. But the explanation

was ultimately found by Pringle who showed that these high rates of oscillation are myogenic: the rapid contraction of one set of muscles is induced by the stretching brought about by their antagonists; whereas shortening of the muscles leads to a rapid relaxation of tension. This same mechanism can explain the high rate of vibration in the timbal muscles of cicadas which may go up to 4,500 Hz.

It has now been shown by Josephson (*J. exp. Biol.*, **59**, 781; 1973) that in the singing katydids *Neoconocephalus* and *Eoconocephalus*, relatively high rates of sound pulses, rising to about 200 Hz, generated by rubbing the wings together, are controlled by synchronous, or neurogenic, muscles in which each contraction is initiated by a nerve impulse. The high rates of contraction are dependent on two factors: a rise in temperature to about 36° C (12° C above ambient temperature) in the thoracic muscles, and a consequent persistence of an unfused tetanus in the muscles at rates of 160 Hz or more.

This mechanism is expensive in energy, for each muscle must contract while its antagonist is still contracting, and the songs may continue for several hours on end. But the heat produced is necessary to maintain the required temperature; and the author suggests that the mechanism may have evolved as the result of sexual selection—the females opting for the males with the highest pulse frequencies in their song.

Stratigraphic record of Early Eocene *Hyopsodus* and the geometry of mammalian phylogeny

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Eocene strata in Wyoming spanning a period of 5 million years record a branching phylogeny which exhibits gradual phyletic evolution, overall size increase with iterative evolution of small species and character divergence following the origin of each new lineage.

THE gradual nature of evolutionary change was a central tenet in Darwin's *Origin of Species*¹. Darwin acknowledged that new forms of life appear suddenly in the fossil record and attributed this to gaps in the record. He stated that the stratigraphical column was probably not sufficiently complete to record in ancient lineages the continuous gradual change through natural selection that he and others had observed in domestic animals under artificial selection. The phylogeny of the condylarth *Hyopsodus* presented in this report is the first detailed record of mammalian evolution at the species level. The record is based on numerous superposed stratigraphical levels within one restricted geographical area of one geological formation: it provides new palaeontological evidence of Darwinian gradualism in evolution.

Phylogeny of *Hyopsodus*

Condylarths are archaic ungulates which are important because they were abundant in the early Tertiary and because the group includes the ancestors of the living Artiodactyla and Perissodactyla. During the past ten years, Yale expeditions have made large collections of fossil mammals from approximately 400 localities in the Early Eocene Willwood Formation^{2,3} of the Big Horn Basin in northwestern Wyoming. *Hyopsodus* is the most common mammal in these collections, being represented by approximately 4,000 jaw fragments containing two or more teeth. Morphologically, *Hyopsodus* had a long body with relatively short limbs (see inset in Fig. 1), suggesting a fossorial habit. In 1965 measurement of a stratigraphical section 1,690 feet (515 m) thick was completed by G. Meyer and L. Radinsky in the Antelope Creek-Elk Creek-Fifteen Mile Creek drainage area which covers a geographical area of approximately 20 km by 60 km, all of which is badlands with little or no cover of vegetation. The measured section spans most of Early Eocene time, a period of approximately 5 m.y.⁴.

The original stratigraphical section included forty-five localities yielding specimens of *Hyopsodus*, and the nearly horizontal attitude of strata in this area permitted interpolation into the original section of an additional ninety localities yielding *Hyopsodus* discovered since 1965. The stratigraphical position of each of these localities was determined to the nearest 20 feet (6.1 m), then the length and width of all lower first molars of *Hyopsodus* were measured and the log of the product of the measurements was computed for each successive interval. The product of length multiplied by width of M_1 is the best dental measurement for characterising the size of closely related mammalian species, and it is in general the best discriminant of closely related species occurring together in any one stratigraphical level⁵. Sympatric species of the same genus living today can only rarely be diagnosed by morpho-

logical differences in their cheek teeth, though the species usually differ significantly in size. Similarly, for the *Hyopsodus* from a given stratigraphical level, size is the only characteristic of the molars which permits species diagnosis.

Size is plotted against stratigraphical position in Fig. 1 for all specimens from the study area containing M_1 (a total of 928). In this plot a number of lineages are apparent, the best defined being those labelled *Hyopsodus latidens*-*H. minor*, *H. lysitensis* and *H. powellianus*. The range of variation of the sample of ninety-two *H. lysitensis* from level 1,360 is typical of living species, and undoubtedly closely approaches the maximum variation in size to be expected in any sample of a species of *Hyopsodus*. Samples at levels 440, 600, 660, and 1,240 have ranges of variation exceeding that expected of one species and the samples have tentatively been divided to indicate this, though they are not clearly bimodal.

The dashed lines in Fig. 1 indicate the limits of variation of each of the well defined lineages, and in addition show the most probable relationships of *H. simplex*, *H. wortmani*, *H. mentalis*, and *H. walcottianus* to the better represented species. It is apparent from the figure that the relatively small species *H. loomisi* gave rise in early Gray Bull time to two species *H. simplex* and *H. sp. A*. *Hyopsodus* sp. A gave rise in late Gray Bull time to *H. latidens* and *H. miticulus*. In the Lysite, *H. miticulus* gave rise to *H. lysitensis* and *H. powellianus*. *H. powellianus* apparently gave rise to *H. mentalis* and *H. walcottianus* at the beginning of Lost Cabin time. *H. minor* and *H. wortmani* are Lysite and Lost Cabin descendants of *H. latidens* and *H. lysitensis*, respectively.

The coexistence of three species of *Hyopsodus* in late Gray Bull, Lysite and Lost Cabin times is confirmed, although the relationships of each of these species are fundamentally different than has been proposed by recent authors. Gazin⁶ and Guthrie⁷, who grouped all specimens into either a lower Gray Bull, an upper Gray Bull, a Lysite or a Lost Cabin sample, proposed (using the species names of Fig. 1) that *H. loomisi* gave rise to a small, a medium and a large species, each of which then continued relatively unchanged through upper Gray Bull, Lysite and Lost Cabin strata. In other words they advocated a small lineage *H. simplex*-*H. minor*-*H. wortmani*, a lineage of medium-sized species *H. latidens*-*H. lysitensis*-*H. mentalis*, and a lineage of large species *H. miticulus*-*H. powellianus*-*H. walcottianus*. Division of the lower Eocene strata into forty-seven levels, instead of the four used by Guthrie and Gazin, shows that only the last group is a true evolutionary lineage. Whereas Gazin and Guthrie portray three lineages, each appearing suddenly and continuing almost unchanged through the remainder of the Early Eocene, it is clear from Fig. 1 that each new species evolved gradually from its parent species. Indeed, no species appeared abruptly, a fact which accords well with Darwin's view.

Each *Hyopsodus* lineage underwent gradual but significant size change. In the *H. latidens*-*H. minor* and *H. lysitensis*-*H. wortmani* lineages this change was sufficient to justify dividing each lineage into two time-successive phyletic species. Plotting the size data on a log scale makes the variability of samples of large and small species comparable but tends to

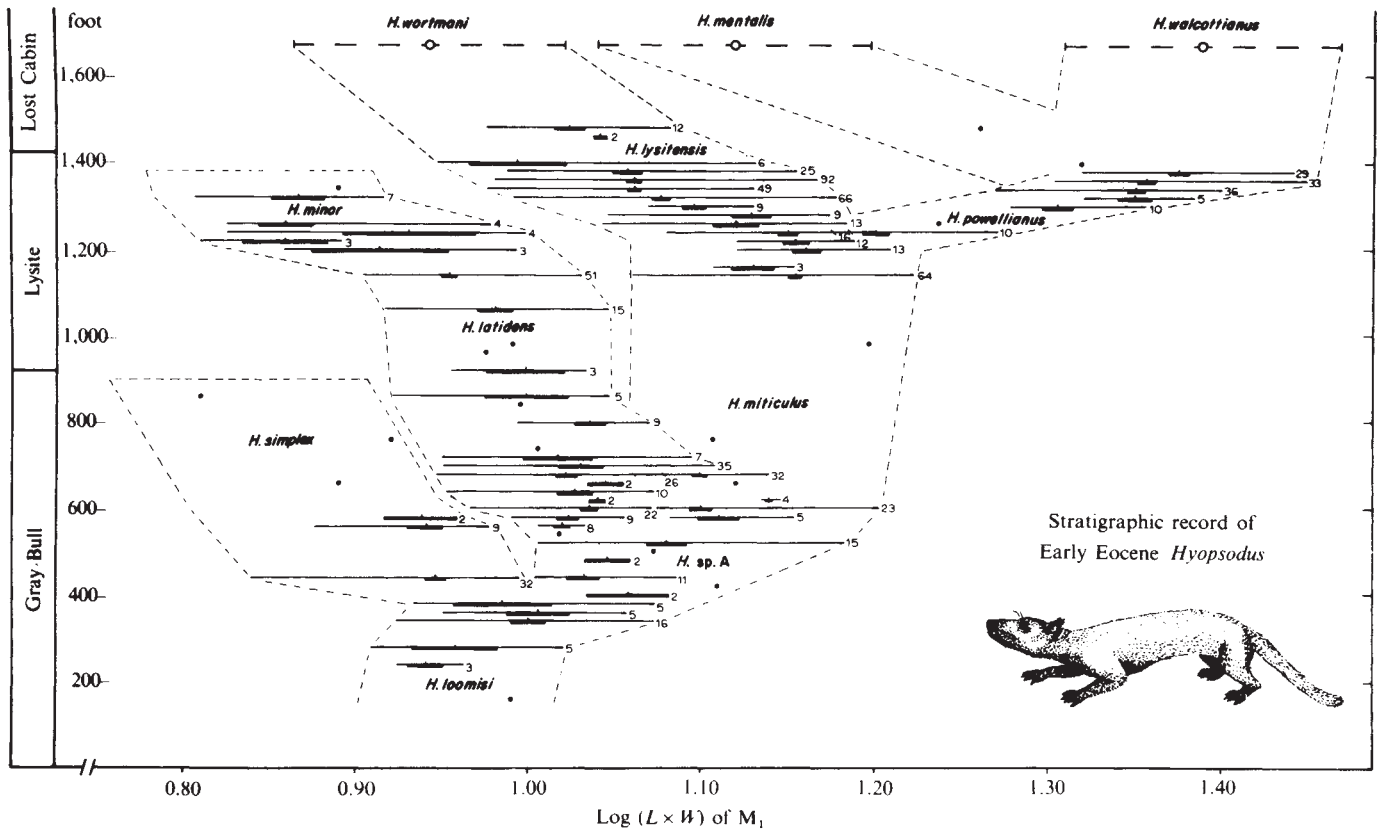


Fig. 1 Stratigraphical record and phylogeny of Early Eocene species of *Hyopsodus*. Abscissa is the $\log_{10}$ of the length (mm) multiplied by width (mm) of the first lower molar. Ordinate is height (feet) above base of Willwood Formation, which includes Gray Bull, Lysite and Lost Cabin faunal zones. Sampling interval is 20 feet (6.1 m); average duration of one 20 foot interval is about 60,000 yr. Vertical slash is mean, heavy bar is standard error of mean, light bar is observed range, small numerals indicate sample size, and filled circles represent single specimens. Open circles and dashed lines at top of figure are means²⁸ and expected ranges for Lost Cabin species from the Wind River Basin which are poorly represented in the Willwood section. Inset, flesh restoration of *Hyopsodus* from Gazin⁶.

obscure the great overall increase in size in this radiation. The *Hyopsodus* radiation clearly conforms to Cope's rule, which states that there is a tendency for animal groups to evolve toward larger physical size (or from small size⁸). Of the eleven *Hyopsodus* species represented, however, six species became smaller and only five larger during the course of their evolution, indicating that Cope's rule does not deterministically describe evolution at the species level.

Iterative evolution, whereby a number of distinctive forms evolve repeatedly from one stem lineage, has been documented in the evolution of lobsters⁹ and in the evolution of pygidium morphology in Cambrian trilobites¹⁰. The same phenomenon is apparent in Fig. 1, where four small species *Hyopsodus simplex*, *H. minor*, *H. wortmani* and *H. mentalis* were derived successively and independently from the larger stem lineage *H. loomisi*–*H. sp. A*–*H. miticulus*–*H. powellianus*–*H. walcottianus*.

Palaeontological evidence such as that presented in Fig. 1 has occasionally been used to support the thesis that species arise sympatrically. In this case, as in earlier cases¹¹, allopatric speciation with subsequent migration may explain the fossil record equally well. It is clear from Fig. 1 that speciation must have preceded size divergence. Presumably speciation in *Hyopsodus* involved the evolution allopatrically of significant hybrid inviability between two populations, followed in sympatry by gradual size divergence to decrease interspecific competition¹².

The length of time observed before two lineages became clearly distinct in size is somewhat surprising. In the best documented case, *H. lysitensis* and *H. powellianus* seem to have required about 40 feet (12.2 m) of section, or on the order of 100,000 yr to become clearly differentiated. This slow, gradual divergence differs considerably from the expected rapid rate of displacement if resource competition between species was the

only controlling factor¹³. Species of *Hyopsodus* are the most common fossil mammals found in the Lower Eocene of North America. They presumably lived in populations of large size, and the slow rate of genetic change in large populations¹⁴ may have retarded the speed with which size displacement could be accomplished.

Geometry of mammalian phylogeny

A persistent gap in the palaeontological evidence for evolution has been the apparent absence in the fossil record of intermediate forms linking two species unequivocally to a common ancestor. Palaeontologists have generally reacted to this absence of intermediate forms by continued search for more complete stratigraphical sections. Recently a new 'punctuated equilibrium' model of animal phylogeny has been advanced^{15,16}, which explains the gaps in the fossil record as a natural result of allopatric speciation. According to this model, new species arise as peripheral isolates of a central population and the isolate, being small, has virtually no chance of being preserved in the fossil record. A successful isolate may replace the central population in a relatively short period of time—instantaneously considering the length of geological time. The postulated pattern of phylogeny resulting from this model includes long periods of time without significant morphological change in a lineage. Rarely this morphological equilibrium is punctuated by a rapid speciation event. The net result, according to the punctuated equilibrium model, is a fossil record of isolated static species without intermediate linking fossil populations.

The punctuated equilibrium model can be tested only by a relatively complete, stratigraphically controlled fossil record such as that of *Hyopsodus* already described. The phylogeny of *Hyopsodus* appeared^{6,7} to conform to the punctuated equi-

brium model and for this reason it was chosen to test the model.

Figure 1 shows clearly that when a large number of stratigraphical levels were studied within the Willwood Formation, the resulting phylogeny of *Hyopsodus* exhibited neither long periods of morphological equilibrium, nor rapid speciation events. Probably the chief reason why interspecies transitions are rare in the fossil record is, as Darwin proposed, because of the incomplete nature of most stratigraphical sections. Local stratigraphical successions that seem to be nearly complete, such as the Willwood Formation section, have still only rarely been studied in detail. In addition to the multi-lineage radiation described in this report, a number of other examples in the literature exhibit phyletic gradualism in single lineages of invertebrates¹⁷⁻²⁰ (although details of these studies have been challenged, for numerous characters gradual evolution remains the most parsimonious interpretation, given the fossil record in each case) and vertebrates²¹⁻²⁵. The punctuated equilibrium model remains to be substantiated by a detailed stratigraphical study within a single geological formation.

The fossil record of *Hyopsodus*, when studied in detail, seems to justify Darwin's view that species evolved gradually. Study of other characters in other phylogenies of species with a good fossil record is needed to determine the extent to which population size, character variability²⁶ and other factors affect the rate of evolutionary divergence following speciation.

The stratigraphical record of human evolution unfortunately still includes many gaps; study of the available evidence in a stratigraphic context²⁷, however, suggests that hominid phylogeny conforms to the pattern of gradual character divergence exhibited here by *Hyopsodus*.

The *Hyopsodus* specimens on which this report is based were collected by Yale expeditions directed by Professor E. L. Simons, and I thank him for access to this collection. I also thank P. Dodson, N. Eldredge, J. Ostrom, D. Pilbeam,

E. Simons, F. Simons, G. Meyer, T. Bown for discussion; M. McKenna, D. Savage for comparative specimens; M. Egan for assistance with Fig. 1. Financial assistance provided by a Shadle Fellowship of the American Society of Mammalogists is gratefully acknowledged.

Received October 8, 1973.

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Photoreactivating enzyme from human leukocytes

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A photoreactivating enzyme has been found in human leukocytes. The specificity of the enzyme for cyclobutyl pyrimidine dimers in DNA may allow direct evaluation of the role of dimers in ultraviolet-induced carcinogenesis in man.

PHOTOREACTIVATION is the specific repair of ultraviolet (220-300 nm)-induced cyclobutyl pyrimidine dimers in DNA¹. The reaction is carried out by the photoreactivating enzyme, which requires light in the range 300-500 nm for its activity^{2,3}. The specificity of the enzyme for pyrimidine dimers enables its use as a diagnostic tool: if ultraviolet-induced biological damage is photoreactivable, pyrimidine dimers are probably a major cause of the damage⁴. This approach has been used to show that dimers are largely responsible for ultraviolet-induced death and mutation in prokaryotes and simple eukaryotes⁵⁻⁷.

Ultraviolet light produces skin cancer in humans, which in the extreme case of xeroderma pigmentosum leads to death⁸. Consequently, it is important to evaluate directly the role of dimers in the induction of such cancers in man. The apparent lack of the photoreactivating enzyme in placental

mammals^{4,9} has prevented direct evaluation of the role of dimers in human carcinogenesis although studies with model systems are in progress¹⁰.

I show here that human cells do have a photoreactivating enzyme. I have purified the enzyme to apparent homogeneity and have studied some of its physicochemical properties.

Radioactive DNAs were prepared as previously described¹¹. The specific activity of the ³²P-labelled T7 DNA was 397 c.p.m. pmol⁻¹ and that of the ³H-thymidine-labelled T7 DNA was 12.5 c.p.m. pmol⁻¹. Pyrimidine dimers were produced by exposure to 254 nm radiation; dimer content of ³H-thymidine-labelled DNA was determined by formic acid hydrolysis, chromatography in *n*-butanol: acetic acid: H₂O (40:6:15), elution and counting of dimer-containing and monomer-containing regions of the chromatogram in a scintillation counter¹². For the ³²P-DNA, the dimer content was determined by (1) digestion of the DNA with DNase I, venom phosphodiesterase and alkaline phosphatase to a mixture of inorganic phosphate, mononucleosides and dimer-containing ³²P-labelled oligonucleotides; (2) adsorption of the labelled oligonucleotides to Norit, and (3) counting in a planchet counter¹¹.

TABLE 1 Summary of purification procedures

Procedure	Fraction	Volume (ml)	Protein (mg ml ⁻¹)	Units (pmol h ⁻¹)	Specific activity (pmol mg ⁻¹ h ⁻¹)
Cell extract	1	2.0	82	400	2.44
Ammonium sulphate pellet	2	1.0	6.2	256	41.4
Isoelectric focusing pool	3	1.0	0.015	95	6,350.0

Photoreactivating enzyme activity was assayed by adding cell extract or purified enzyme to 0.2 ml 0.02 M potassium phosphate buffer, pH 7.2, containing 0.1 mM dithiothreitol, 0.1 mM EDTA and 30-100 pmol of ultraviolet irradiated, ³²P-labelled T7 DNA. For each determination one assay tube was placed in the dark and another was exposed to photoreactivating light from a General Electric 150 W spot lamp. The dimer content of the samples was then determined as described above. Photoreactivating activity is the difference in dimer content of the light and dark samples; units of photoreactivation are pmol⁻¹ mg⁻¹ h⁻¹. Protein concentrations were determined by the Lowry method¹³, using bovine serum albumin as the protein standard.

The enzyme

Plasmagel (Bellon Labs) (4 ml) was added to 12 ml of freshly drawn human peripheral blood treated with 170 U of heparin. The suspension was mixed gently and allowed to settle 45 min at 37°C. The supernatant suspension, from which most of the erythrocytes had settled, was centrifuged at 2,000 r.p.m. in the SS-34 rotor of the Sorvall RCB-2. The cells were washed three times by gentle resuspension in 2 mM potassium phosphate buffer, pH 7.2, containing 0.15 M NaCl and centrifugation as before. Washing removed serum proteins and proteins added with the Plasmagel. The cells

TABLE 2 Characterisation of the photoreactivating enzyme activity

Enzyme	Ultraviolet exposure to ³² P-labelled T7 DNA (erg mm ⁻²)	Photo-reactivating light exposure (min)	Photo-reactivating enzyme activity (pmol h ⁻¹)
Fraction 3	3,000	0	0.1
Fraction 3	3,000	30	10.3
Fraction 3, trypsin treated, 34°C 5 min	3,000	30	6.53
Fraction 3	0	30	0.12
None	3,000	30	0.02

were suspended in 2 ml of buffer E (10 mM Tris, pH 7.0, 0.1 mM EDTA, 0.1 mM dithiothreitol) and sonicated for 45 s in a Kontes sonicator. The resulting solution was fraction 1. Solid ammonium sulphate (0.163 g ml⁻¹ of fraction 1) was added and the mixture was stirred gently for 15 min on ice. The solution was centrifuged for 15 min at 15,000 r.p.m. in the SE 12 rotor of the Sorvall. Less than 30% of the photoreactivating enzyme activity appeared in the pellet; to the supernatant was added 0.163 g (per ml of fraction 1) of ammonium sulphate; the mixture was stirred 15 min after all the ammonium sulphate had dissolved, and centrifuged in the SE12 rotor as before. All the enzyme activity appeared in the pellet, which was dissolved in 1 ml buffer E (fraction 2). Fraction 2 was layered into the middle of an isoelectric focusing gradient (110 ml) prepared as follows: the cathode was placed at the bottom of the column. The cathode solution contained 14 ml H₂O, 0.2g NaOH and 12g sucrose. Sample of 55 ml of a 110 ml linear density gradient (formed by mixing in a LKB gradient apparatus a solution of 47.8 ml glycerol, 5 µl 0.1 M EDTA, 5 µl 0.1 M dithiothreitol, 9.83 ml H₂O and 1.88 ml LKB pH 3.5-10 ampholine solution with a solution of 57.4 ml H₂O, 5 µl 0.1 M dithiothreitol and 0.625 ml of the ampholine solution) was layered into a 110 ml jacketed isoelectric focusing column maintained at 4°C by a Lauda circulator. Fraction 2 was layered onto the gradient solution, and the rest of the gradient solution was then added. The anode solution (0.1 ml phosphoric acid, 10 ml H₂O) was layered on top of the gradient. A voltage of 300 V was applied across the column for 72 h. Fractions (0.5 ml) were collected and assayed for photoreactivating enzyme activity. Although several small peaks of enzyme were detected at pH values greater than 5.4, most of the enzyme was located at pH 5.4. The peak fractions were combined to yield fraction 3. The purification procedure is summarised in Table 1.

As Table 2 shows, the purified enzyme requires ultraviolet-irradiated DNA and photoreactivating light for its activity. The enzyme preparation must be added to the DNA for the reaction to proceed. The most important property of the enzyme is its ability to monomerise pyrimidine dimers in DNA. Since the ³²P-DNA assay for photoreactivation is indirect, I tested the ability of the enzyme preparation to reverse dimers in ³H-thymidine-labelled DNA. In this case both dimers, TT and CT, and the monomer, T, can be isolated and measured directly. Figure 1 shows that in the presence of photoreactivating light the enzyme preparation causes dimers to disappear from the ³H-thymidine-labelled DNA. In the dark the dimer content decreases very little, if at all; thus

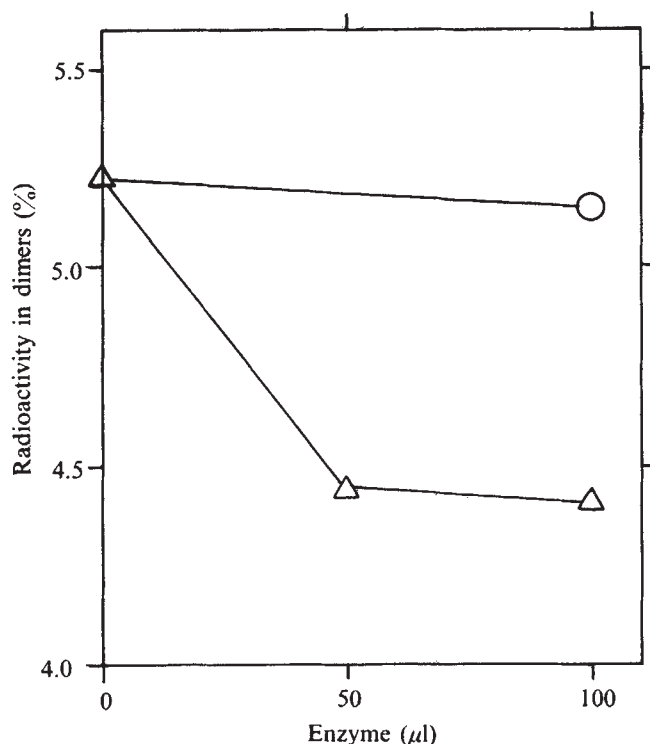


FIG. 1 Photoreactivation of dimers in ³H-thymidine-labelled DNA. Fraction 2 enzyme was added to 1.55 nmol of dimer-containing ³H-thymidine-labelled DNA in a standard assay mixture. After 5 min incubation at 37°C in the dark, one sample (○) was kept in the dark while the others (△) were exposed to 30 min of photoreactivating light. The dimer content of the samples was determined by the method of Setlow *et al.*¹².

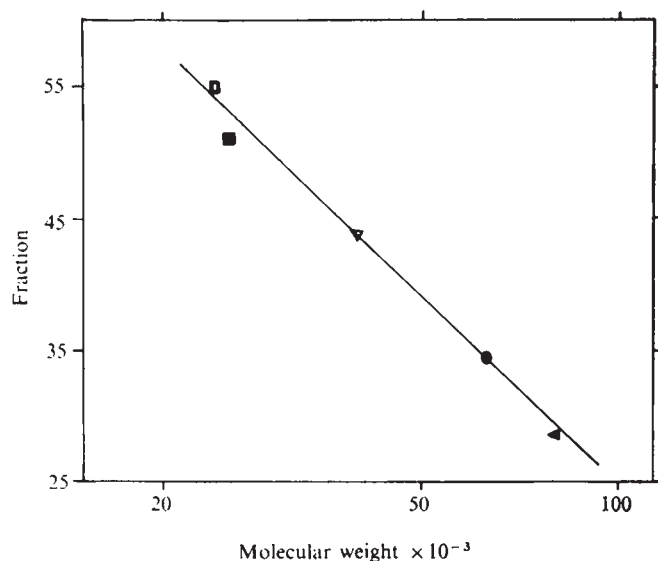


FIG. 2 Molecular weight determination. The elution position on a 27×1.1 cm column of Biogel P-100 in buffer E + 1.0M NaCl of protein standards (bacterial alkaline phosphatase (▲), horse haemoglobin (●), cytochrome c dimer (■) and trypsin (□) was determined. The elution position of the human photoreactivating enzyme (▽) was used to estimate its molecular weight at 40,000.

the disappearance of dimers from DNA in the light is due to true monomerisation rather than excision. The demonstration of photoreactivation by the 32 P-DNA assay also indicates that excision did not occur, as this assay does not distinguish excised dimers (in short pieces of DNA) from those remaining in high-molecular-weight DNA¹¹.

Since several types of low molecular weight molecules sensitise monomerisation of dimers¹⁴⁻¹⁶, it was important to show that the species in this preparation responsible for dimer monomerisation has a protein component. Treatment of the enzyme with 10 mg trypsin for 1 and 5 min at 34° C decreased enzyme activity to 85 and 59% of its original value, respectively. In this time the treatment at 34° C without trypsin did not affect enzyme activity. It is thus likely that the molecule responsible for photoreactivation has a protein component.

The enzyme has an apparent molecular weight of 40,000 (Fig. 2). The isoionic pH of the enzyme is 5.4, which agrees qualitatively with the rapid migration towards the anode of the enzyme on polyacrylamide gels carried out under non-denaturing conditions (see below).

As with the *E. coli* photoreactivating enzyme, the pH optimum of the human enzyme is 7.2. At pH values 8.1 and 6.8 the enzyme showed 29 and 49% of the activity exhibited at pH 7.2. However, the human enzyme is inhibited by high ionic strength and at $\mu = 0.18$, the optimum for the *E. coli* enzyme, the human enzyme has only 20% of the activity it has at $\mu = 0.05$.

The final enzyme preparation, fraction 3, yields one major protein band on electrophoresis under non-denaturing conditions (Fig. 3). That this band is photoreactivating enzyme was shown by slicing a gel into 0.5 cm lengths, eluting the protein by electrophoresis from the gel and assaying each fraction for photoreactivating enzyme activity. The major band of enzyme activity coincided with the major protein band on the gel. In addition, two smaller peaks of enzyme activity appeared in the regions 0-1 and 0.3-0.4 of the gel. Since fraction 3 enzyme is isolated from a narrow band of the isoelectric focusing column, it is unlikely that these smaller peaks represent multiple enzyme species; rather they are probably aggregates of the enzyme into dimer and higher multiples. Such aggregates have also appeared in glycerol gradients performed in conditions of low ionic strength.

Photoreactivation in mammalian cells

Although photoreactivation is found in all phyla and in many types of cells⁴, data on the presence of the photoreactivating enzyme in placental mammals have been contradictory¹⁷⁻²². The problems in interpreting these data are, (1) for positive results, to distinguish between true cases of enzymatic photoreactivation and effects due to photoprotection, and (2) for negative results, to differentiate true absence of the enzyme from inadequate exposure or insensitive assay procedures. Clearly photoreactivation is more difficult to demonstrate *in vivo* in mammalian cells than in other cells; whether this is due to the low cell content of the enzyme or to the inaccessibility of the DNA to the enzyme is not clear. Cook⁴ reported an example of the latter: cell cultures of the South American woolly possum (*Caluomys derbianus*) Tasmanian potoroo (*Potorus tridactylis*) and opossum (*Didelphis marsupialis*) (all marsupials) had detectable photoreactivating enzyme when assayed *in vitro* whereas *in vivo*, they required very long exposures (6 h) to photoreactivate 40% (potoroo) and 65% (woolly possum) of their dimers. But if inaccessibility of DNA had been the cause, Cook and McGrath⁹ should have detected enzyme activity in placental mammals *in vitro*. These are two possible explanations for their failure to do so. First, although they assayed various tissues, they did not test mammalian leukocytes, in spite of the high activity they found in toad leukocytes. Second, they may not have been able to detect the enzyme if it had been present. Unlike the yeast and *E. coli* enzymes, the human enzyme is inhibited at high ionic strength, and at

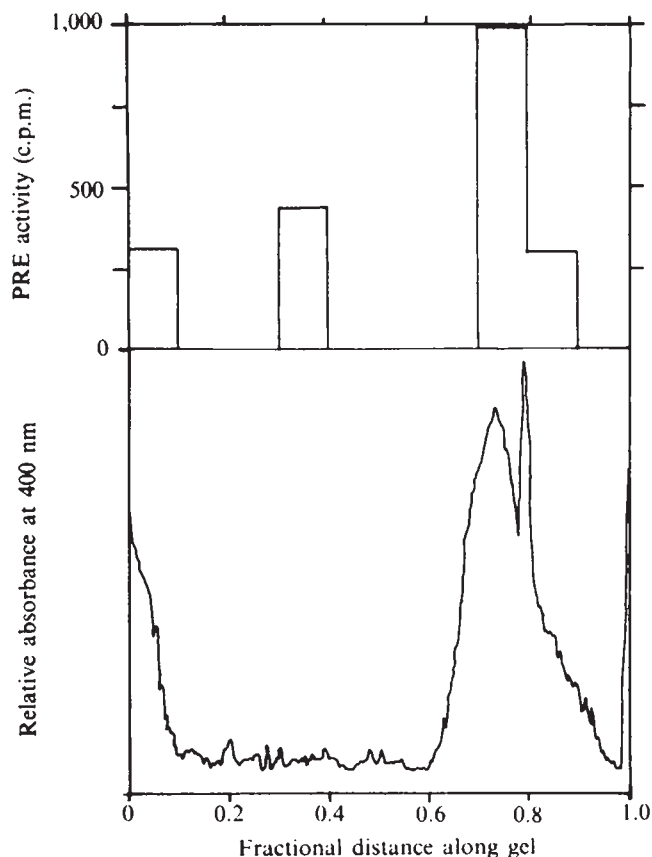


FIG. 3 Absorbance and photoreactivating activity profiles of fraction 3 enzyme. The 5% gels were prepared according to the method of Davis²³. Two gels were prepared: after electrophoresis, one was stained in Coomassie blue and, after destaining, was scanned in a Gilson spectrophotometer for determination of protein distribution. The other gel was sliced into 0.5 cm segments; the enzyme was eluted by electrophoresis and assayed for photoreactivating enzyme activity as usual. The peak at 0.81 is tracking dye.

the ionic strength used by Cook and McGrath (0.11) it would have been only 35% as active as under optimal conditions ($\mu = 0.05$).

Photoreactivation is of primary biological interest as an analytical tool. Since the photoreactivating enzyme acts specifically on cyclobutane-type pyrimidine dimers, true enzymatic photoreactivation of ultraviolet-induced biological effect indicates that pyrimidine dimers were the molecular alteration responsible for the damage. Such tests have shown that dimers are a primary cause of death and mutation in prokaryotic and eukaryotic cells⁶⁻⁷.

Since sunlight induces cancer in man, it is tempting to speculate that pyrimidine dimers are the molecular cause of such cancers. But so far, it has not been possible to test this hypothesis directly and model systems have been necessary: Hart and Setlow presented data indicating that ultraviolet-induced, potentially tumorigenic lesions in the fish *Poecilia formosa* can be photoreactivated¹⁰. But now it should be possible to select a human or other placental mammalian cell type and photoreactivation conditions optimal for the direct evaluation of the role of pyrimidine dimers in the induction of cancerous growth by ultraviolet light.

I thank Professor G. A. Granger and members of his laboratory, Dr R. Daynes, P. Runge, J. Kramer and T. Jeffes, for help in obtaining and advice on handling the leukocytes; Professors M. J. Chamberlin and J. C. Sutherland for helpful discussions, and R. Knauft for technical assistance. This work was supported by grants from the National Institute of Health and California Cancer Research Coordinating Committee.

Received October 25, 1973.

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Specialised transformation in *Escherichia coli* K12

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A specialised transformation system has been developed in E. coli K12 by using specialised transducing phage DNA as donor DNA. Transformation of a marker on a F' episome has also been achieved.

GENERALISED transformation for chromosomal markers in *Escherichia coli* K12 has been reported recently¹⁻⁴. These studies demonstrated two major factors in determining the transformability of *E. coli*: CaCl_2 treatment of the cell surface to allow permeation of DNA and an appropriate intracellular status of various DNases, especially the ATP-dependent (*recB-recC*) DNase⁵⁻⁸. In this, as in other bacterial generalised transformation systems, the chromosomal donor DNA which is used is heterogeneous with respect to molecular size and genetic information. A transformation system in which essentially all the donor DNA is homogeneous in its genetic composition and its molecular size would be advantageous for studying various aspects of transformation, especially for studying molecular mechanisms of recombination. DNA from specialised transducing *E. coli* phages seems to be the ideal source of transforming donor DNA for this purpose because of the well characterised DNA structure and the relative ease of obtaining a large quantity of pure DNA which is unique for a specific region of the chromosome.

We report here such specialised transformation of a recipient chromosomal as well as an episomal (F') genetic marker. This was demonstrated by using donor DNA isolated from phages $\phi 80\text{pt}^{9,10}$ and λpt^{11} , which carry tryptophan genes, to transform a tryptophan-requiring strain to tryptophan prototrophy.

Isolation of transforming DNA from *E. coli* (Hfr C6) was described previously⁴. The phage DNA was isolated by a phenol procedure¹² after purifying the phages by CsCl density centrifugation. The DNA preparations were extensively dialysed against 1/10 SSC (15 mM NaCl — 1.5 mM $\text{Na}_2\text{citrate}$), and were heated at 70° C for 10 min before use. Shearing of the DNA was carried out with a Sorvall Omni mixer. Transformation was performed as described previously⁴, except that the reaction was stopped by the addition of an equal volume of cold 50 mM sodium citrate. Bacterial and phage strains and all the mutant markers are described in Table 1. In the text only the relevant mutations of each strain will be indicated.

Specialised transformation

Tables 2 and 3 show typical results of experiments involving specialised transformation of a *trp* mutation (*trpB*) with $\phi 80\text{pt}$ (*trpA*⁺*B*⁺*C*⁺) and λpt (*trpA*⁺*B*⁺*C*⁺*D*⁺*E*⁺) DNA. The

TABLE 1 Bacterial and phage strains used in this study

(A) <i>E. coli</i> K12 strains		Relevant genetic characteristics used in this study*	Source
Strains	Derived from		
M0639	JC7623	<i>recB21 recC22 sbcB15 leu-6 trpB9579</i>	This laboratory†
M0671	M0639	<i>recB21 recC22 sbcB15 leu-6 trpB9579 tonB</i>	This laboratory†
M0668	M0639	<i>recB21 recC22 sbcB⁺ leu-6 trpB9579</i>	This laboratory§
M0649	M0639	<i>recB⁺ recC⁺ sbcB15 leu-6 trpB9579</i>	This laboratory§
M0650	M0639	<i>recB⁺ recC⁺ sbcB⁺ leu-6 trpB9579</i>	This laboratory§
M0640	JC7623	<i>recB21 recC22 sbcB15 leu-6 ΔtonBtrpABCDE</i>	This laboratory
M0646	M0640	<i>F'(trpB9579 tonB⁺ attφ80⁺)/M0640</i>	This laboratory¶
M0868	CH56	<i>ΔtonBtrpABCDE recA1</i>	This laboratory**
(B) Bacteriophage strains		Source	
Strains			
φ80pt(<i>trpA⁺B⁺C⁺</i>)		H. Ozeki	
φ80pt(<i>trpA⁺B⁺C⁺</i>)		This laboratory††	
φ80pt(<i>trpD⁺E⁺</i>)		F. Imamoto	
φ80		F. Imamoto	
λpt(60-3 <i>trpA⁺B⁺C⁺D⁺E⁺O⁺</i>)		F. Imamoto	
λcI26		R. J. Huskey	

* Besides the genetic characteristics listed here, M0639, M0671, M0649 and M0640 have the following markers: *his-4 ara-14 thr-1 thi-1 lacY-1 ml-1 xyl-5 galK2 proA2 argE3 str-31 tsx-33 sup-37 amber. In addition to the above markers, M0650 is *his⁺* and M0668 is *his⁺ thy⁻*.*

† Constructed by transformation with DNA from W3110 (*trpB9579*)¹³ provided by F. Imamoto.

‡ *tonB* mutation introduced by mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine.

§ Constructed by conjugation with Hfr KL16.

|| Constructed by transformation with DNA from strain CH56 provided by W. Maas.

¶ Original F' is described in ref. 14. Before transfer to M0640, the *trpB9579* mutation was introduced and the *lac* genes were deleted.

** Constructed by mating with Hfr KL16-99.

†† Isolated from phages released from a φ80pt(*trpA⁺B⁺C⁺*) lysogen of W3110 (*trpB9579*)¹³.

φ80pt (*trpA⁺B⁺C⁺*) used as donor DNA carries a part (*trpABC*) of the *trp* operon of the *E. coli* chromosome, in place of the *int-red* region of the φ80 genome. Therefore, the φ80pt is *int⁻ red⁻* and *trpA⁺B⁺C⁺* (ref. 15). This *int⁻ red⁻* condition of φ80pt eliminates the phage recombination systems which might have complicated studies of the host recombination system. For similar reasons, we have also used DNA from λpt(*trpA⁺B⁺C⁺D⁺E⁺*), a plaque-forming phage λ containing the entire *trp* operon of *E. coli* in place of at least the *int-red* region of the bacteriophage λ chromosome as in φ80pt. As Table 2 shows, when CaCl₂ treated cells of the multiauxotrophic transformable *E. coli* K12 strain (M0671 *recB21 recC22 sbcB15 trpB9579 leu-6 tonB*) were exposed to unshredded, intact DNA isolated from φ80pt(*trpA⁺B⁺C⁺*), considerable numbers of Trp⁺ transformants appeared on the selective plates, but no Leu⁺ transformants were detected. The same degree of specialised transformation for *trpB* was also observed with sheared φ80pt(*A⁺B⁺C⁺*) DNA which had an average molecular weight of 8×10^6 , approximately a quarter the size of a φ80 genome (31×10^6)¹⁶. Fig. 1 shows the profile of transforming activities of the sheared φ80pt DNA after sucrose gradient centrifugation, showing that the transforming activity observed with the sheared DNA is not due to transformation by any residual intact φ80pt DNA

TABLE 2 Transformation of the *trpB* mutation with φ80pt and λpt DNA

Source of DNA	No. of transformants	
	Trp ⁺	Leu ⁺
None	0	0
φ80pt (<i>trpA⁺B⁺C⁺</i>)	1,808	0
φ80pt (<i>trpA⁺B⁺C⁺</i>), sheared	1,814	2
λpt (<i>trpA⁺B⁺C⁺D⁺E⁺</i>)	1,158	0
λpt (<i>trpA⁺B⁺C⁺D⁺E⁺</i>), sheared	840	0
<i>E. coli</i> Hfr C6 (<i>trp⁺ leu⁺</i>), sheared	106	144

This transformation was performed as described previously⁴ with a slight modification (see text) using M0671 as the recipient cells. The concentrations of the DNA used were 5 μg per 0.5 ml for φpt and λpt DNA and 10 μg per 0.5 ml for *E. coli* DNA. The average molecular weight of sheared DNA was 8.5×10^6 , 8.9×10^6 and 10×10^6 for φ80pt, λpt and *E. coli* DNA, respectively. The number of transformants is presented as the sum of the Trp⁺ or Leu⁺ colonies produced with a total of 2.2×10^7 viable recipient cells.

molecules. A slight shift of the activity from the peak of the absorbance profile of the quarter molecules toward the higher molecular weight side is probably due to the unique effect of the molecular weight of donor DNA in the transformation of *E. coli*; maximum transforming activity was

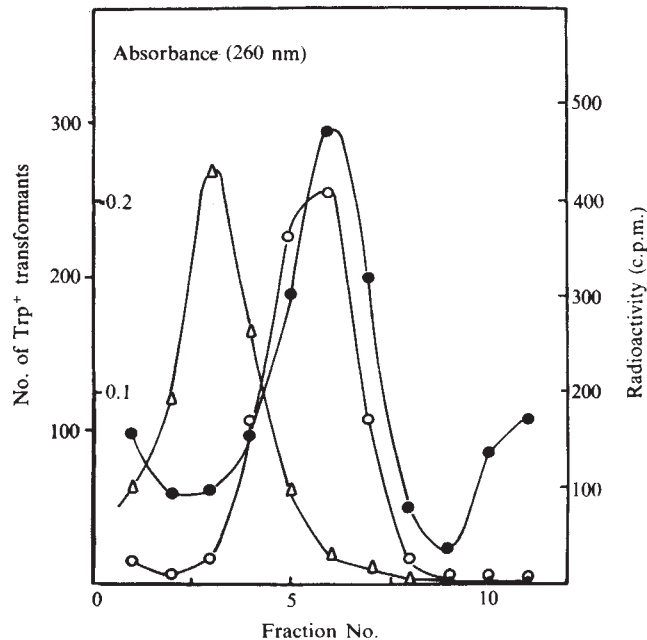


FIG. 1 Sucrose gradient centrifugation of sheared φ80pt DNA. Fifty μg of sheared φ80pt(*A⁺B⁺C⁺*) and ³H-φ80 DNA (0.05 μg, reference marker) in 0.5 ml of 1/10 SSC were applied to an 11.5 ml gradient of 5–20% sucrose in a buffer of 20 mM Tris-HCl, pH 7.6, 1 M NaCl and 1 mM EDTA. The centrifugation was performed at 36,000 r.p.m. for 5 h at 20°C using a Spinco SW41 rotor. Fractions (1 ml) were collected, a part (0.2 ml) of each fraction was withdrawn for radioactivity assays and the rest was dialysed against 1/10 SSC. Transformation was performed as described previously⁴ using 0.1 ml of the dialysed fractions as donor DNA and M0671 as a recipient. The number of transformants is the sum of Trp⁺ colonies produced with a total of 8×10^6 recipient cells. Δ, ³H-φ80 DNA; ○, transformants; ●, A_{260 nm}.

observed with DNA the molecular weight of which was between 1×10^6 and 2×10^6 (refs 3 and 4). Essentially the same results were obtained with unsheared and sheared λ pt(*trpA*⁺*B*⁺*C*⁺*D*⁺*E*⁺) DNA (Table 2).

Table 3 shows that the transforming activity increased linearly up to 5 μ g DNA per tube (0.5 ml) then levelled off, more or less the same pattern as observed when chromosomal DNA was used. The maximum transformation frequency with these DNA preparations was 2×10^{-5} to 8×10^{-5} . This is ten to thirty times greater than that observed with *E. coli* chromosomal DNA (1×10^6 to 5×10^{-6})¹⁻⁴. This higher efficiency is to be expected, since the concentration of the *trpB* gene in the ϕ 80pt DNA (also λ pt DNA) preparation was approximately eighty times greater than that in the chromosomal DNA preparation. The observed lower transformation efficiency than expected (eighty-fold increase) may have been due to recipient base sequence non-homology with the phage-specific base sequences which lie adjacent to the *trp* genes in the donor DNA. The biologically active material in the DNA preparation was sensitive to DNase (Table 3), eliminating the possibility that transduction by contaminating intact phages was the source of transformants. A ϕ 80 resistant (*tonB*) mutant strain was used as recipient for most experiments as a precaution to exclude such a possibility.

As Table 4 shows, the DNA from other types of ϕ 80 and λ phages, ϕ 80pt(*trpA*⁺*B*⁺*C*⁺), ϕ 80pt(*trpD*⁺*E*⁺), wild type ϕ 80 and λ , did not give any Trp⁺ transformants, clearly indicating that the presence of the *trpB*⁺ gene in the donor DNA preparation is the source of the transformation of the recipient's *trpB*⁻ marker. We next investigated the effect on specialised transformation of the genetic background of the recipient cells with respect to *recB* *recC* (ATP-dependent DNase) and *sbcB* (exonuclease I)¹⁷ genes, because generalised transformation of *E. coli* was shown to be affected by these genes¹. As we found in that system, the highest specialised transformation efficiency by ϕ 80pt(*A*⁺*B*⁺*C*⁺) DNA was observed in recipient strain M0639 (*recB21 recC22 sbcB15*) in which both DNases were missing. A small but significant number of transformants, approximately 20% of that observed with M0639, was obtained with M0649 (*recB*⁺ *recC*⁺ *sbcB15*).

TABLE 3 Transformation of the *trpB* mutation with various concentrations of DNA

DNA	Concentration (μ g per 0.5 ml)	No. of Trp ⁺ transformants
None	0	0
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺)	1	140
	2	213
	5	458
	10	544
	10 (+DNase)	1
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺), sheared	1	334
	2	557
	5	1,090
	10	1,017
	10 (+DNase)	2
λ pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺ <i>D</i> ⁺ <i>E</i> ⁺)	1	171
	2	167
	5	273
	10	603
	10 (+DNase)	2
λ pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺ <i>D</i> ⁺ <i>E</i> ⁺), sheared	1	180
	2	231
	5	700
	10	714
	10 (+DNase)	1

Transformation was performed as described previously⁴ with a slight modification (see text) using M0671 as the recipient cells. The average molecular weight of sheared DNA was 8.5×10^6 and 8.9×10^6 for ϕ 80pt and λ pt DNA, respectively. DNase was added at a concentration of 10 μ g per tube (0.5 ml). The number of transformants is presented as the sum of the number of Trp⁺ colonies produced with a total of 7×10^7 viable recipient cells.

TABLE 4 Transformation of the *trpB* mutation with DNA from various sources

Source of DNA (genotype)	No. of transformants
None	0
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺)	1012
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺), sheared	1333
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺)	1
ϕ 80pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺), sheared	1
ϕ 80pt(<i>trpD</i> ⁺ <i>E</i> ⁺)	0
ϕ 80pt(<i>trpD</i> ⁺ <i>E</i> ⁺), sheared	0
ϕ 80	0
ϕ 80, sheared	0
λ pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺ <i>D</i> ⁺ <i>E</i> ⁺)	648
λ pt(<i>trpA</i> ⁺ <i>B</i> ⁺ <i>C</i> ⁺ <i>D</i> ⁺ <i>E</i> ⁺), sheared	770
λ (c126)	0
λ (c126), sheared	3

Transformation was performed as described previously⁴ with a slight modification (see text) using M0671 as the recipient cells and DNA at a concentration of 5 μ g per tube (0.5 ml). The average molecular weight of sheared DNA from various phages was in the range of 8×10^6 to 9×10^6 . The number of transformants is the sum of Trp⁺ colonies produced with a total of 1.9×10^7 viable recipient cells.

Transformation occurred to a much lesser extent, approximately 5% of that with M0639, with B0650 (*recB*⁺ *recC*⁺ *sbcB*⁺). No transformants were obtained with M0668 (*recB21 recC22 sbcB*⁺), which is recombination-deficient. The absence of transformation in the *Rec*⁻ recipient indicates the participation of the host recombination system in the transformation process.

Mechanism of transformation

We have characterised the nature of the transformants obtained in our specialised transformation system. All the 120 tested Trp⁺ transformants obtained with sheared DNA were sensitive to ϕ 80 and did not produce any phage particles even after ultraviolet irradiation. Almost all (117 out of 120) the Trp⁺ transformants obtained with unsheared DNA were also sensitive to ϕ 80 and unable to produce phage after irradiation with ultraviolet light. These ϕ 80-sensitive Trp⁺ transformants produced by sheared and unsheared DNA were stable in their acquired Trp⁺ character. We could not detect any Trp⁻ segregants among more than 5,000 progeny grown from representative Trp⁺ colonies in a non-selective medium. We also tried to determine whether some Trp⁺ transformants still possessed the original *trp*⁻ markers in addition to the newly acquired Trp⁺ character. The *trp* operon and the *cysB* gene are known to be about 50% linked by cotransduction with P1 phage. The studies on specialised transduction had shown that integration events could place the newly added *trp*⁺ marker sometimes proximal and sometimes distal to the *trp*⁻ marker with respect to *cysB* (ref. 15). In the latter case, at least, it would be possible by P1 transduction readily to transfer the *trp*⁻ marker linked to *cysB* and without the *trp*⁺ marker. Twenty P1 lysates were prepared from random ϕ 80-sensitive Trp⁺ transformants (ten each from Trp⁺ transformants obtained with sheared and non-sheared DNA) and transduction was performed with a *trp*⁺ *cysB*⁻ recipient. All Cys⁺ transductants (more than 5000 Cys⁺ were tested) were Trp⁺, suggesting that the pre-existing *trp*⁻ marker was not present and had been replaced by a *trp*⁺ marker in all twenty transformants. These results, plus the fact that the transformation can be accomplished with sheared DNA suggest that the mechanism of transformation of the *trp* marker does not involve addition of the entire ϕ 80pt genome to the *E. coli* chromosome, but rather the replacement of the pre-existing *trp*⁻ marker by the *trp*⁺ marker present in the ϕ 80pt donor DNA. This is similar to what occurs in generalised transformation in *E. coli* and is in sharp contrast to specialised transduction

TABLE 5 Transformation of the *trpB* mutation on F' episome

Strain	Relevant genotype	Source	DNA	Concentration (μg per 0.5 ml)	Transformation frequency (trp^+ per 10^6 cells)
M0646	<i>F' tonB⁺trpB⁻/ΔtonBtrpABCDE</i>	None	$\phi 80\text{pt}(\text{trpA}^+\text{B}^+\text{C}^+)$	0	<0.1
				1	7.4
				2	18.1
				5	56.7
				10	51.1
		$\phi 80\text{pt}(\text{trpA}^+\text{B}^+\text{C}^+)$, sheared		10 (+DNase)	<0.1
				1	20.3
				2	30.7
				5	63.7
				10	29.3
		$\lambda\text{pt}(\text{A}^+\text{B}^+\text{C}^+\text{D}^+\text{E}^+)$		10 (+DNase)	<0.1
				10	73.3
				10 (+DNase)	<0.1
		$\lambda\text{pt}(\text{A}^+\text{B}^+\text{C}^+\text{D}^+\text{E}^+)$, sheared		10	78.1
				10 (+DNase)	0.4
M0640	<i>ΔtonBtrpABCDE</i>	None		0	<0.1
				$\phi 80\text{pt}(\text{A}^+\text{B}^+\text{C}^+)$	<0.1
				$\phi 80\text{pt}(\text{A}^+\text{B}^+\text{C}^+)$, sheared	<0.1
				$\lambda\text{pt}(\text{A}^+\text{B}^+\text{C}^+\text{D}^+\text{E}^+)$	<0.1
				$\lambda\text{pt}(\text{A}^+\text{B}^+\text{C}^+\text{D}^+\text{E}^+)$, sheared	<0.1
				10	<0.1

Transformation was performed as described previously⁴ with a slight modification (see text). Because of the temperature sensitivity of the F' replication, the cells (M0640 and M0646) were grown at 35°C and the selective plates were incubated at 30°C after transformation. The molecular weights of sheared DNA used here were 8.5×10^6 and 8.9×10^6 for $\phi 80\text{pt}$ and λpt DNA, respectively. DNase was added at a concentration of 10 μg per tube (0.5 ml). Trp^+ transformants were scored and the transformation frequency was calculated according to the total number of 9.4×10^6 and 2.7×10^7 recipient cells produced by M0640 and M0646, respectively.

with $\phi 80\text{pt}^{15}$ and to transformation with $\lambda\text{dgal DNA}^{18}$, in which the entire $\phi 80\text{pt}$ and λdgal phage genomes are integrated into the host chromosome.

Transformation of *trp*⁻ on F' DNA

We have also accomplished specialised transformation of a *trpB* mutation located on episomal F' DNA instead of on the chromosome. A transformable strain (M0646) was constructed harbouring an F' which carried the *trp* operon (with a *trpB9579* mutation) plus adjacent chromosomal regions including the *tonB* locus, and with the *trp* operon and *tonB* locus deleted from the chromosome. When this strain was used as recipient for $\phi 80\text{pt}(\text{A}^+\text{B}^+\text{C}^+)$ and $\lambda\text{pt}(\text{A}^+\text{B}^+\text{C}^+\text{D}^+\text{E}^+)$ donor DNA, Trp^+ transformants emerged (Table 5). The frequency of the transformation (5×10^{-6} – 10×10^{-6}) was somewhat lower than the frequency obtained with the same donor DNA but with the recipient's *trpB9579* allele located on the chromosome. Since the F'-bearing recipient cells (M0646) contained a large deletion on the chromosome, from the *tonB* locus through the entire *trp* operon, the Trp^+ transformants seemed likely to be the products of recombination between the homologous *trp* regions on the F'DNA and the $\phi 80\text{pt}$ donor DNA. This was confirmed by our findings that (1) no Trp^+ transformants were obtained when we use recipients (M0640) harbouring the same large chromosomal deletion but lacking the episome (Table 5), and (2) the Trp^+ character could be transferred from Trp^+ transformants to a recombination deficient F' strain (M0868 *recA1 ΔtonBtrpABCDE*) together with other characters associated with the F', such as normal chromium resistance determined by the *tonB* locus^{19,20}. The basic characteristics of the system (Table 5) seem to be the same as those obtained with specialised transformation of the chromosomal *trp* marker.

Value of system

We have described, the transformation of a genetic marker (*trp*) on the *E. coli* chromosome or an F' episome by DNA isolated from specialised transducing phages carrying the *trp*⁺ marker. Although the frequency of the transformation ($\sim 10^{-5}$) is still somewhat lower than that obtained in generalised transformation with other bacterial species, for

example *Bacillus subtilis*, this system provides certain special advantages for studying various aspects of the transfer of genetic information, especially the mechanism of genetic recombination. The homogeneity of the genetic and molecular structure of donor DNA, stemming from its specialised transducing phage origin, is unique for bacterial transformation systems. The extensive genetic and biochemical background knowledge of *E. coli* and its related phages will be another advantage to this system. Of course, specialised transformation is not limited to *trp* genes but should also be applicable to any other well-characterised genes in *E. coli* such as *gal* or *bio* genes whose specialised transduction by λ phage is well established. Transformation of F' DNA with phage DNA reported here will provide a system in which not only the donor DNA but also the recipient DNA is composed of well defined relatively small molecular weight molecules. Such a system may be particularly useful in future biochemical and biophysical studies to examine the structural changes occurring in both recipient DNA and donor DNA during genetic recombination.

We thank S. Orenstein for technical assistance and J. Tamayo for preparing the manuscript. We also thank Dr P. Margolin for critically reading the manuscript and for discussions. This work was supported by a grant from the US National Science Foundation to M.O. S.D.C. was supported by a US Public Health Science research fellowship. M.O. is a US Public Health Service research career development awardee.

Received October 19; revised December 6, 1973.

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Membrane model for the circadian clock

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The biological clock may be a feedback system involving ions and ion-transport channels.

RHYTHMS which will entrain to a 24 h light/dark cycle and persist under constant conditions with a period of about 1 d are apparently ubiquitous among eukaryotic organisms¹. A striking feature of these circadian rhythms is that the 'free-running' period, exhibited when there is no entrainment by environmental cycles, remains close to 24 h over a wide range of temperatures. (The 'free-running' period is implied in all subsequent references to the period, unless specified otherwise. There is a survey of circadian rhythms in ref. 1.) Most investigators believe that these rhythms are generated by a cellular biochemical system and that, though the details may vary from species to species, some fundamental mechanism is common to all.

Nevertheless, the molecular elements of this biological clock have proved elusive. To facilitate the search for the oscillatory system, it is helpful to consider models. Two conceptually different approaches have emerged. One conceives of the clock as a 'tape' with discrete biochemical events occurring in a strict sequence. In molecular terms, this has been interpreted as sequential gene expression: transcription of DNA proceeds, one gene at a time, at a controlled rate, as described most explicitly in the chronon model of Ehret and Trucco². Another approach, pursued by Pavlidis³⁻⁵, Pye⁶ and Sel'kov⁷, conceives of the clock as a biochemical network with self-sustained oscillations arising from feedback within the biochemical system. This notion can be traced to the oscillator theories of Pittendrigh and Bruce⁸ and the experimental work of Chance and associates^{9,10}.

Our model incorporates the network idea, but identifies ions and membrane-bound ion transport elements with the biochemical clock, and thus with the primary oscillations. Feedback arises from the effect of ion concentrations on the activities of the ion transport structures, which in turn affect ion distributions. (Obviously changes in ion concentrations imply changes in trans-membrane ion gradients and vice versa. However, the regulatory effects of ions relate most immediately to concentrations while ion fluxes are most readily discussed in terms of gradients. Therefore we use both descriptions, although they are equivalent.) The rate-limiting kinetics—either of ion transport or of change in transport activity—are postulated to be a function of the lipids, which in natural membranes are known to be adapted to the environmental temperature^{11,12}. Temperature compensation of the free-running period is hypothesised to be a consequence of this. Light affects the clock by open-

ing a membrane ion gate which may itself be photosensitive or be triggered hormonally from a remote photoreceptor.

Three concepts on which this model is based thus deal with the role of ions and membranes, the effect of light, and the importance of membrane lipids. To provide a more complete picture, we propose that the circadian change in membrane transport activity may involve the migration of membrane-intercalated particles within the plane of the membrane, and that time-dependent cooperative phenomena may account for accurate timekeeping and homeostasis.

Role of ions and membranes

Chemical agents which might alter the phase or period have been sought in the hope of pinpointing the molecular components of the clock. A few have been known for some time (D_2O ¹³, ethanol¹⁴, arsenite and *p*-chloromercuribenzoate¹⁵), but these gave no clear indication of a unique target. More recent experiments implicate ions and membranes. Valinomycin (a highly specific carrier of K^+) causes phase shifts in two systems, the bean plant *Phaseolus*¹⁶ and the dinoflagellate *Gonyaulax*¹⁷. Ethanol may act by changing the ionic permeability of biological membranes¹⁸; ethanol effects have now been demonstrated in *Phaseolus*¹⁴, *Gonyaulax*¹⁷ and the isopod *Euxirolana*¹⁹. Although ion pulses are ineffective in several systems, this may be attributable to regulatory mechanisms for maintaining ion balances. K^+ pulses do phase shift the rhythm in isolated eyes of the sea hare *Aplysia*²⁰ and lithium lengthens the period in the plant *Kalanchoe*²¹.

Ion involvement is also indicated by the fact that phytochrome, which may act as a K^+ gate^{22,23}, apparently mediates the effect of light on the free-running period in *Phaseolus*²⁴ (28.1 h in red, 24.7 h in far red, 26.3 h in darkness or light of other wavelengths). Electric fields, which have an effect on some rhythms²⁵, should only affect spatially ordered systems, such as membranes and their surrounding charge distributions.

Rhythms are phase shifted by light pulses and entrained by light cycles, and the free-running period usually varies with the constant light intensity²⁶. In our model, light acts by perturbing ion concentrations (probably K^+ , since K^+ and valinomycin pulses phase shift like light). The photoreceptors coupling to rhythmic systems are hypothesised to be photosensitive ion gates, as in visual systems and plant phytochromes. This allows for close coupling between the photoreceptor and the clock in unicellular organisms; specifically, a K^+ flux through the photoreceptor gate augments or depletes the K^+ concentration directly. In higher systems,

hormones may mediate between a specialized photoreceptor and the other cells²⁷. Consequently, the coupling hormone should induce a permeability change (possibly specific for K^+) in its target membrane. In support of this is Rensing's finding that the moulting hormone ecdysone phase shifts the rhythm in isolated *Drosophila* salivary glands²⁷. In *Chironomus* salivary glands, ecdysone acts by increasing the K^+/Na^+ ratio in cell nuclei²⁸. Circadian rhythm photoreceptors can be very sensitive and fast-acting, characteristics typical of photosensitive ion gates. A 1 ms flash phase shifts the fungus *Pilobolus*²⁹; an extraretinal photoreceptor of the sparrow is sensitive to 0.1 lux of green light³⁰. Winfree has found 1,000 ergs cm^{-2} applied over 2 min to be sufficient to saturate phase shifting in the fruit fly *Drosophila*²¹.

If the clock is a feedback oscillator with ions on one hand and membranes on the other, it must involve circadian changes in transmembrane ion fluxes. These fluxes, both passive and active, should be mediated primarily by membrane proteins. Activity changes in these proteins may involve either synthesis and degradation or activation and inhibition. Since protein synthesis is probably not required to drive the clock oscillation (see later), the clock function presumably involves activation and inhibition of transport proteins. The kinetics of the clock then must reflect both the kinetics of this activation and inactivation and the kinetics of the transport processes *per se*. But these rates probably depend also on the characteristics of the membrane lipids.

According to the 'fluid mosaic' concept of membrane structure³², membrane proteins are intercalated into the lipid bilayer and can move in the plane of the membrane through the fluid lipid matrix. Because the kinetics of this protein migration will depend on the fluidity of the membrane lipids, this migration constitutes a lipid-dependent process for activation and inactivation of membrane protein functions. A mechanism for temperature compensation of circadian rhythms is immediately suggested. The lipid composition of membranes is controlled to maintain a definite fluidity independent of temperature^{11,12}. The kinetics of protein migration should depend on the viscosity of the medium, so lipid adaption should render this temperature-compensated. The limited data available³³ suggests that the activities of some membrane-bound enzymes are temperature-compensated by the lipid adaption phenomenon, so the kinetics of protein-mediated ion transport may also be compensated in this way. Lipid adaptation also compensates the rate of passive ion diffusion through the lipid bilayer³⁴.

Characteristics of rhythms

It has long been known that observed rhythmic processes may not be an integral part of the timekeeper. Specifically, their suppression does not affect the clock. The only unmistakably clock-related experiments are those that concern shifting the phase or changing the period of the rhythm. Only those data will be considered in the ensuing discussion of clock properties.

A 24 h period is not easily derived from the relatively rapid processes encountered in biochemistry. The chronon model² relies on a 24 h transcription and recycling sequence, but the biochemistry has not been sufficiently elucidated to evaluate the model's kinetic feasibility. A biochemical oscillator might have difficulty with 24 h periods; experimentally studied systems have periods of no more than 15 min⁶ so a 100-fold frequency reduction is necessary. To circumvent this problem, Pavlidis has suggested that coupled oscillators may oscillate as a group with periods much longer than the individual units⁵. Spatial inhomogeneity in the timekeeper reactions may also lengthen the period. However, such a large frequency reduction should be critically dependent on the coupling parameters, and it is difficult to

imagine a biochemical system capable of maintaining the coupling constants within the limits required for an accurate period. The membrane model, by drawing on slower processes, such as lateral diffusion of proteins within the membrane³⁵⁻³⁷, can achieve 24 h periods more easily.

To provide for temperature compensation of the period, the chronon model² postulates aqueous diffusion processes to be rate-limiting, but the negative temperature coefficients shown by the algae *Oedogonium*³⁸ and *Gonyaulax*³⁹ are not accounted for. The network model of Pavlidis and Kautzmann⁴ postulates reduced enzyme levels at higher temperatures, attributing negative temperature coefficients to overcompensation. However, it cannot readily explain dual mode temperature compensation, such as that seen in *Euglena*⁴⁰, which has a period-length Q_{10} of 1.0 when grown autotrophically, but a Q_{10} of 0.93 when grown mixotrophically. The membrane model can attribute this to the fact that fatty acids of *Euglena* lipids vary with culture conditions⁴¹; negative temperature coefficients may be a consequence of overcompensation in the lipid adaptation process.

Circadian rhythms can be phase shifted by temperature steps and entrained by temperature cycles. (By convention, a temperature step is a prompt change in the environmental temperature. A temperature pulse involves a step followed (usually 12 h later) by an opposite step returning the system to its initial temperature. The repeated application of temperature pulses (usually with a 24 h period) is called a temperature cycle.) In *Drosophila*, entrainment by 24 h temperature cycles follows directly from the form of the phase response curve for single temperature steps⁴². A temperature cycle apparently acts as a series of independent steps, implying that phase shifting due to a temperature step is completed within 12 h. Experimentally, the direction of the temperature change determines the direction of the phase shift. For a temperature step up, a phase advance occurs as if the clock had run fast for awhile; for a step down, a phase delay occurs^{40,42-47}. This indicates that the temperature compensation process requires several hours to complete. In our membrane model, the lipids must adjust to a change in temperature with a time constant on the order of hours. Lipid compensation requires about 4 h in stationary phase *E. coli*⁴⁸, and earthworms injected with an extract from cold-adapted earthworms change their lipid saturation within 3 h⁴⁹.

In some systems, the overt rhythm is lost at low temperatures and the mechanism seems to settle into a unique phase point; when the temperature is raised, the rhythm resumes with a phase defined by that time^{39,46,50}. The membrane model provides an interpretation: the temperature is below the adaptation range for the lipids in the membrane, which then will undergo a transition to a more condensed phase. This should cause the ion gradients to fall into the pattern characteristic of the unique phase point; it is interesting that in *Gonyaulax* both low temperature and bright light hold the system at the same point, CT 12. (Circadian time (CT) is defined by dividing the period into twenty-four equal portions. CT 0 is chosen as the beginning of the light period or its equivalent in a free-running system.)

To analyse the effects of light on rhythms, a simple two-parameter example is helpful. Assume that the rhythm involves just one ion and its associated transport pathways. The trans-membrane ion gradient, x , will oscillate as in Fig. 1a. Membrane transport, y , will also vary but, for simplicity, let it have only two states (Fig. 1b). Light has the effect of depleting the ion gradient since it opens an ion gate. Therefore, a light pulse dropping the gradient to x_0 may have two effects. Applied before CT 18, it will cause a phase delay because the membrane is in the active mode and x will repeat its accumulation. A light pulse given after CT 18 will cause a phase advance since the membrane is in the passive mode and x will skip ahead in its discharge.

This simple illustration will not give quantitative agreement with phase shifting data. For a proper analysis of the two-dimensional oscillator, the dynamical equations should be written as $dx/dt = f_1(x, y)$ and $dy/dt = f_2(x, y)$. Light of intensity, L , acts on the system to deplete the gradient x . Since this is a passive flux, it should proceed at a rate proportional to x and to some function of the light intensity $f_3(L)$. Therefore, dx/dt must be modified to include the light effect: $dx/dt = f_1(x, y) - x f_3(L)$. An analysis of a particular set of equations of this type quantitatively reproduces the effects of light on the *Drosophila* system including the influence of the light intensity on the period^{3,4}. While these equations are not unique in their ability to account for the data, they do establish the quantitative plausibility of this sort of system. Of course, any network model can be fit to these equations, but the phase shifting caused by the rapid destruction of x is particularly descriptive of the depletion of ion gradients.

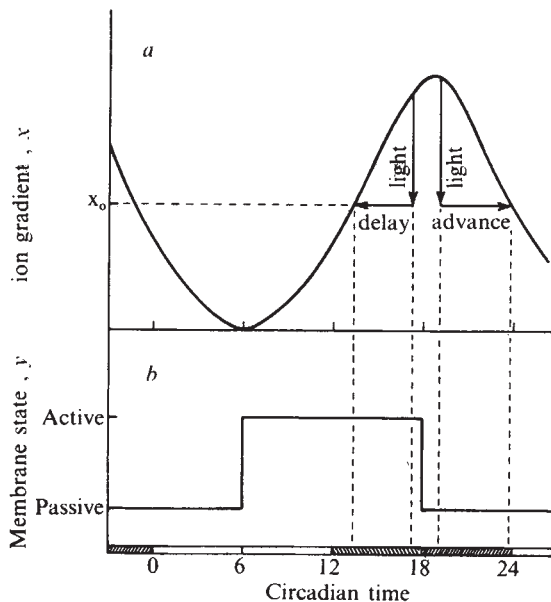


FIG. 1 Hypothetical oscillations in a simple one-ion system with only two membrane states. A light pulse applied just before CT 18 results in a phase delay since it brings the ion-membrane system to a state equivalent to that at about CT 13. Similarly, an identical light pulse just after CT 18 results in a phase advance. Maximum phase shifts are obtained at night.

The cellular clock couples biochemically to diverse expressed rhythms possessing various phase angles. Control of some processes, such as leaf movement in *Albizia*, can be readily attributed to ions²³, and rhythms in membrane processes such as photosynthesis, respiration, phototaxis and excitability could depend directly on ion variations. Some rhythmic processes, such as bioluminescence in *Gonyaulax*, involve, in part, variations in enzyme and substrate levels^{51,52}. Reconciling this with a membrane model is more difficult, although variations in enzyme levels could be achieved through ion-mediated activation and inactivation, without *de novo* synthesis. However, ions might also act by stimulating the production of inducers—perhaps for all rhythmically expressed proteins, perhaps only for specific rhythmically-expressed RNA polymerases. A diurnal variation in polymerases has been reported⁵³. The induction hypothesis allows genes to be regulated in groups, which would facilitate control of several complex processes associated with one circadian rhythm. Furthermore, this would make mRNA synthesis a driven rhythm rather than a part of the clock itself, explaining the failure of RNA synthesis inhibitors to phase shift the rhythm.

Speculations and implications

In considering the detailed structure of the membrane, we could imagine a simple model in which passive and active transport channels respond directly to changes in ion concentrations so that they flip back and forth into on and off modes. This probably has too fast a response time and would generate rapid oscillations as observed in mitochondria⁵⁴. Instead, we have suggested a requirement for lateral diffusion of protein in the membrane^{55,56}. Particles seen by freeze-fracture electron microscopy exhibit characteristic arrangements and sizes in differentiated membrane regions⁵⁵⁻⁵⁹ correlated with pH and other ion concentrations^{37,55,60,61}. We propose that these particle distributions can be regulated by oscillating ion concentrations and may themselves then regulate the ion concentrations to drive the oscillation. This is easy to imagine for structures such as cell junctions; ATPases and other enzymatic functions might also be activated and inactivated in this way. Furthermore, this process of constructing particle arrays should be relatively slow.

At the risk of conveying a simplistic and overly specific image, we present in Fig. 2 a diagrammatic version of our membrane timekeeper in the style of Singer and Nicolson's 'fluid mosaic' membrane³². This shows changes in both particle arrangements and sizes, caused by the ion distributions. The state of these particles, in turn, determines the direction and activity of membrane transport. While only one ion and a corresponding set of transport proteins is represented, other ions and other transport systems may be tied in, forming a system of coupled oscillators. A photoreceptor which serves as a K^+ gate is included in this picture; in other cases, this might be a hormone-triggered ion gate.

Circadian rhythms persist in enucleate cells^{62,63} and seem to be unaffected by many nucleic acid and protein synthesis inhibitors^{50,64-67}. Among such inhibitors only cycloheximide has been shown to affect a circadian rhythm⁶⁸ although the period change was small considering the degree of protein synthesis inhibition. The fact that inhibition of protein synthesis does not have a larger and more universal effect suggests that the clock keeps time relatively independently of precise enzyme levels. This is particularly compatible with the membrane model, since it does not require precisely balanced enzyme activities, especially to achieve temperature compensation.

A minimal dependence on stochastic fluctuations in enzyme levels has adaptive advantage in a clock mechanism and is an example of the principle of homeostasis: circadian clocks are insensitive to the perturbations they normally encounter⁶⁹. Time-dependent cooperative phenomena provide an attractive means for achieving timekeeping precision and homeostasis. The sigmoid curve characteristic of cooperative phenomena provides a more precise definition of time and is less sensitive to fluctuations in enzyme, metabolite or threshold levels than are functions based on non-cooperative processes. Indeed, regulation in biological systems is commonly associated with cooperativity^{70,71}. The formation of particle arrays ought to show time-dependent cooperativity, since it should be a nucleation process analogous to crystal growth.

A model should provide insight for further research. Our model makes specific and testable statements about the ionic nature of the fundamental oscillator, the action of light, the role of lipids in temperature effects, the insensitivity of the clock to fluctuations in enzyme levels, and the role of membrane-intercalated particles in maintaining self-sustained oscillations in ion concentrations.

In clock mutants, altered proteins might be involved in ion transport or lipid metabolism. Phenotypically, mutants might have altered ion transport properties, defects in membrane particle aggregation, or differences in lipid fluidity.

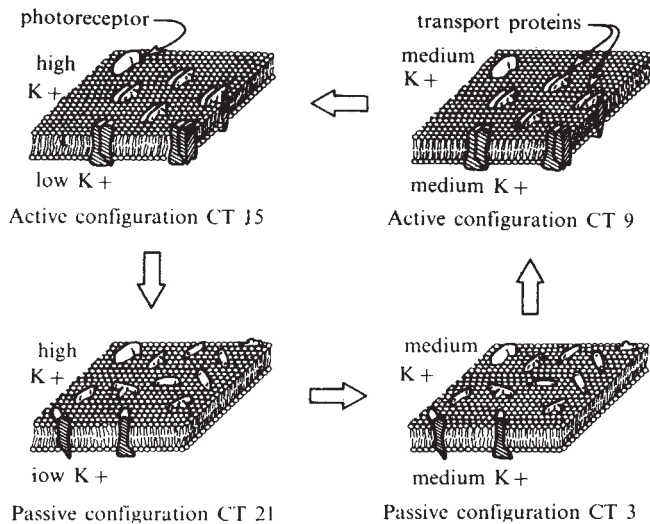


FIG. 2 Schematic representation of the way in which a fluid mosaic membrane³² might keep time. This illustrates the simple one-ion oscillator discussed earlier.

Membrane lipids can also be changed nutritionally or by inhibiting the lipid adaptation mechanism and changing the temperature. Changes in the period should correlate with changes in the lipids; the time course of lipid compensation in rhythmic cells should be determined.

This model predicts that the photoreceptor is membrane-bound and functions as an ion-gating device. In unicellular organisms, phase shifting with light should cause a change in the ion distributions. Mediating hormones in higher animals should produce changes in the ionic permeabilities of their target membranes. The response of membrane transport systems to ion concentrations *in vitro* can be analysed to see whether these systems are capable of self-sustained oscillations with 24 h periods.

Ferritin labelling and freeze-fracture electron microscopy should be useful in observing circadian rhythms in the arrangement of membrane-intercalated particles.

In summary, we have described a membrane model for the biological clock, which accounts qualitatively for many of its characteristic features. Ion concentrations and ion transport channels function as a feedback system to generate self-sustained circadian oscillations. Light acts on the rhythm either directly or through hormonal coupling to deplete trans-membrane ion gradients. Temperature compensation of the free-running period is a consequence of the temperature adaptation of membrane lipids. We have speculated that the timekeeping involves time-dependent cooperativity and the rearrangement of membrane-intercalated particles.

We thank Drs M. W. Karakashian, N. R. Krieger, M. D. McDaniel, D. Mergenhagen, R. E. Schmitter and B. M. Sweeney for stimulating discussions and criticism. This work was supported by a grant from the National Institutes of Health.

Received October 29; revised December 6, 1973.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Infrared observations of Comet Kohoutek before perihelion passage

I REPORT in this letter broadband observations at 10 μm and 20 μm of the nucleus of Comet Kohoutek. These measurements were taken about 1 week before perihelion, while the comet was about 0.35 AU from the Sun. Table 1 summarises the results, which show that the comet was an intense infrared object, magnitude about -5 , and that the nuclear material responsible for the infrared emission heated up rapidly as the comet sped toward the Sun.

10- μm magnitude to be -4.56 relative to α Boo. (The extinction correction at 10 μm was 0.15 mag per air mass.) The uncertainty in my results arises primarily from the uncertainty in the standard magnitudes for α Boo and α Sco and also from the temporal variation in the infrared extinction. I estimate both these effects to contribute an error of no more than ± 0.03 mag in the final results.

Table 1 shows that as the comet grew brighter at about 10 and 20 μm , the colour difference decreased as expected from solar heating of the solid material in the nucleus. This colour change is reflected in the sharp rise of the colour temperature. The derived colour temperatures are above those expected from a grey-body sphere heated by sunlight at the same solar distance as the comet. (For example, at 0.33 AU the expected temperature is about 525 K.) Similar

TABLE 1 Infrared magnitudes of Comet Kohoutek

Date	[10 μm]	[20 μm]	[20 μm - 10 μm]	Colour temperature (K)	R(AU)*
December 19, 1973 1800 h UT	-4.51	-5.54	-1.03	500	0.39
December 20, 1973 1800 h UT	-4.74	-5.60	-0.86	700	0.36
December 21, 1973 1800 h UT	-5.03	-5.80	-0.77	750	0.33

* Taken from the Comet Kohoutek ephemeris of D. K. Yeomans.

I obtained these measurements with the 1.3-m telescope at Kitt Peak National Observatory. The infrared detector was a germanium bolometer with a beam size of 24 arcs. The chopping secondary of the telescope was adjusted to give a beam separation of 38 arcs in declination. During the observations, I centred the beam on the position of peak infrared emission; this location defined the infrared nucleus of the comet. The magnitudes in Table 1 refer only to the infrared flux originating from the portion of the comet within the beam's area. As I found the comet's infrared emission extended over an area greater than 1 arc min, the comet's total integrated infrared magnitudes must be greater than those listed in Table 1.

All magnitudes are relative to the standard star α Boo. Since α Sco was conveniently close to the comet on the dates of observation, I also compared the comet to this star. The advantage of using α Sco was that it suffered from essentially the same atmospheric infrared extinction as the comet, so little correction in the relative magnitudes was necessary. I adopted -4.82 as the 20- μm magnitude of α Sco¹, and I measured its

excessive infrared colour temperatures have been noted for Comet Ikeya-Seki² and Comets Bennett and Tago-Sato-Kosaka³. This colour temperature anomaly is probably due to the spectral behaviour of the absorptive efficiency of the radiating grains in the nucleus². The data in this letter are not sufficient to identify the character of the grains but observations made as the comet streaks from the Sun may answer this important question.

I acknowledge the help of Dr D. E. Kleinmann.

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Received January 3, 1974.

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Detection of methyl cyanide in Comet Kohoutek

Most current models of comets assume that the nucleus is an icy solid body containing fairly complex parent chemical compounds which sublimate and dissociate to provide the radicals and simple molecules that have been observed in cometary spectra¹. Optical spectra show the presence of CN, CO⁺, CH, NH, OH and many others²; proposed parent molecules have included H₂O, HCN, HNCO, CH₃CN and H₂CO³. Previous attempts to detect H₂CO and H₂O were unsuccessful, probably due to excessive beam dilution^{4,5}. Here we report the first direct detection of a parent molecule, methyl cyanide (CH₃CN), at a wavelength of 2.7 mm in Comet Kohoutek (1973f). At the same time we were unable to detect either CN or CO at similar wavelengths.

Observations were made with the NRAO 11-m radio telescope at Kitt Peak, Arizona. The antenna had a half-power beamwidth of 67 × 63 arc s and a main beam efficiency of 0.55 for these observations. The first stage of the receiver is a double sideband Schottky-barrier crystal mixer with a 1,390 MHz IF; the overall system temperature is about 1,450 K single sideband. Spectral line data were taken using three filter banks: 256 contiguous 250 kHz filters, 50 contiguous 250 kHz filters, and 50 contiguous 100 kHz filters; all filter banks had the same centre frequency. The receiver was calibrated with a rotating chopper wheel; the calibration was checked by observing spectral lines of CO and CN with known strength in the galactic sources W51 and DR21. All data were reduced with the NRAO advanced on-line computing system⁶.

Table 1 is a summary of the observations. All values shown

sideband. After correction for the geocentric velocity expected, we calculated the rest frequencies given in the first two lines of Table 2. We are confident that the emission is from the comet nucleus only, as spectra from several days at different equatorial coordinates and different geocentric velocities all show the lines. Also, no lines were seen when we observed one arc min north, south, east and west of the nucleus. On December 3 we observed the same right ascension and declination as on December 1 to check for background sources; this also produced negative results. Finally, no emission was seen at several points along the comet's tail. Searches for CN and CO emission at several positions showed no detectable signals; the best upper limits are given in Table 2.

The two lines that we observed can be identified with 6,3 to 5,3, and 6,0 to 5,0 vibrationally excited transitions of CH₃CN at 110,709.55 and 110,712.22 MHz (ref. 7). Our calculated rest frequencies agree perfectly in line separation but are systematically 0.2 MHz high, or 0.54 km s⁻¹ low in geocentric velocity. This difference is probably not significant in view of the quoted errors. Methyl cyanide has previously been observed in Sgr A and Sgr B where its column density is comparable to that of CN (ref. 8).

The observed antenna temperatures may be used to calculate the optical depth and hence the column density of methyl cyanide. The exact lifetime of the CH₃CN molecule until photo-dissociation, and thus the distance from the cometary nucleus over which it should exist, are not known; a reasonable approximate value is 10⁴ km (refs 3, 9, 10). At a geocentric distance of 1.33 AU the region would subtend approximately 21 arc s and fill 0.06 of the antenna beam. The observed antenna temperature T_A is related to

TABLE 1 Observations of Comet Kohoutek (1973f)

UT Date (1973)	RA	1973.9 Dec.	Geocentric velocity (km s ⁻¹)	Geocentric distance (AU)	Heliocentric distance (AU)	Frequency range observed (MHz)
December 1	13 h 41 min	-20°10'	-34.1	1.36	0.87	(110,691-110,755) (113,472-113,536)
December 2	13 h 47 min	-20°37'	-33.2	1.34	0.85	(112,471-112,535) (115,251-115,315)
December 3	13 h 54 min	-21°06'	-32.1	1.32	0.83	(110,691-110,755) (113,472-113,536)
December 4	14 h 01 min	-21°34'	-31.0	1.30	0.81	(112,471-112,535) (115,251-115,315)
December 5	14 h 09 min	-22°02'	-29.8	1.29	0.79	(110,690-110,754) (113,471-113,535)

are averages over about 6 h of observation time daily. The ephemeris was supplied by T. Clark (Goddard Space Flight Centre) and calculated from elements published in IAU Circular No. 2588. The position of the comet was checked with an optical telescope attached to the antenna; it agreed with star positions from the FK4 catalogue within 10 arc s. The radio data were taken in a beam-switching mode—alternating integrations for 30 s on the source and on a position 30 arc min greater in right ascension. Two frequency ranges were covered simultaneously since the receiver responds to both sidebands. About half the data were taken looking through the fabric dome surrounding the telescope in order to protect the telescope surface from solar heating; the dome loss, about 40%, was taken into account when averaging the individual spectra.

Figure 1 shows spectra from the 100 kHz filter banks on December 1, December 5 and on both days combined; two lines are clearly visible in emission. They appear somewhat broadened by the filter response and by artificial smoothing. These lines were also detected in both 250 kHz filter banks. Also shown is the expected position of the CN line in the upper sideband. A shift in the local oscillator frequency showed that the observed lines were in the lower

the optical depth τ by

$$T_A = B\eta_B T_C \{1 - \exp(-\tau)\} \simeq B\eta_B T_C \tau \quad (\tau \ll 1) \quad (1)$$

where B is the beam dilution factor, η_B is the antenna main beam efficiency, and T_C is the temperature of the cometary gas. T_C is given by

$$T_C = \left(\frac{mc^2}{2k} \right) \left(\frac{\Delta\nu}{\nu} \right)^2 \quad (2)$$

where m is the mass of the molecule, c is the velocity of light, k is Boltzmann's constant and $\Delta\nu$ is the line half-width at $1/e$ intensity at the frequency ν . For $\Delta\nu = 0.12 \pm 0.04$ MHz and $\nu = 110,710$ MHz, $T_C = 263 \pm 160$ K for CH₃CN; the equilibrium temperature for a black body at a heliocentric distance of 0.83 AU is 306 K. Substituting into equation (1) $T_C = 263 \pm 160$ K, $T_A = 0.6 \pm 0.1$ K, $B = 0.06$, and $\eta_B = 0.55$ we obtain $\tau = 0.07 \pm 0.04$ for the 6,0 to 5,0 line. Similarly, the optical depth of the 6,3 to 5,3 transition is $\tau = 0.05 \pm 0.03$ and the CN and CO upper limits are $\tau \leq 0.06$ and $\tau \leq 0.15$, respectively, assuming the same T_C .

TABLE 2 Results

Observed rest frequency (MHz)	Molecule	Transition	Antenna temperature (K)	Intrinsic line half-width at 1/e intensity (km s ⁻¹)	Observed position
110,709.7 ± 0.2	CH ₃ CN	6,3 → 5,3	0.4 ± 0.1	0.32 ± 0.11	nucleus
110,712.4 ± 0.2	CH ₃ CN	6,0 → 5,0	0.6 ± 0.1	0.32 ± 0.11	nucleus
110,712.4	CH ₃ CN	6,0 → 5,0	≤ 0.25		tail
113,491.0	CN	1 → 0	≤ 0.20		(5' from nucleus)
115,271.2	CO	1 → 0	≤ 0.50		nucleus

The optical depth τ for a symmetric rotor such as CH₃CN is³

$$\tau = \frac{16\pi^2 N f_v h^{3/2} \mu^2 v^2 B C^{1/2}}{3c \Delta\nu (kT_C)^{5/2}} \frac{\{(J+1)^2 - K^2\}}{(J+1)} \cdot \exp(-W/T_C) \quad (3)$$

where h is Planck's constant, N is the column density (molecules cm⁻²), f_v is the fraction of molecules in the same vibrational state ($\sim 1/2$), B and C are the molecular rotation constants (both 9,199 MHz), μ is the dipole moment (3.92×10^{-18} e.s.u. cm), and W is the sum of vibrational and rotational energies of the upper level divided by Boltzmann's constant ($W = 500$ K). For the 6,0 to 5,0 transition, $\tau = 2.3 \pm 1.6 \times 10^{-18}$ N, and thus $N = 3.0 \pm 2.1 \times 10^{16}$ molecules cm⁻² averaged over the cloud diameter of 2×10^4 km. The 6,3 to 5,3 transition is theoretically weaker by a factor of 0.75, which agrees within errors with the observed ratio of 0.67. This transition gives $N = 2.9 \pm 2.1 \times 10^{16}$ molecules cm⁻².

Adopting the fluid dynamic model in which the molecular density is proportional to r^{-2} out to a critical radius r_c ,

where the molecules are destroyed⁴, then the number fraction b of CH₃CN molecules is given by

$$b = \frac{N v r_0}{4ZR^2} \quad (4)$$

where v is the molecular velocity (4×10^4 cm s⁻¹), Z is the comet's gas production rate in molecules cm⁻² s⁻¹ rad⁻² and R is the radius of the icy nucleus. Substituting typical values of $Z = 3 \times 10^{18}$, $R = 2 \times 10^6$ cm and $r_0 = 10^9$ cm, we find for CH₃CN that $b = 0.025 \pm 0.018$.

In conclusion, the detection of methyl cyanide in the nucleus of Comet Kohoutek at a reasonable abundance has provided the first direct confirmation of the parent-daughter molecule hypothesis. Future observations of this comet as it passes perihelion are most desirable and should show changes in the density of such heavy molecules and their dissociation products.

The National Radio Astronomy Observatory is operated by Associated Universities, Inc. under contract with the National Science Foundation. The National Astronomy and Ionospheric Center is operated by Cornell University under contract with the National Science Foundation.

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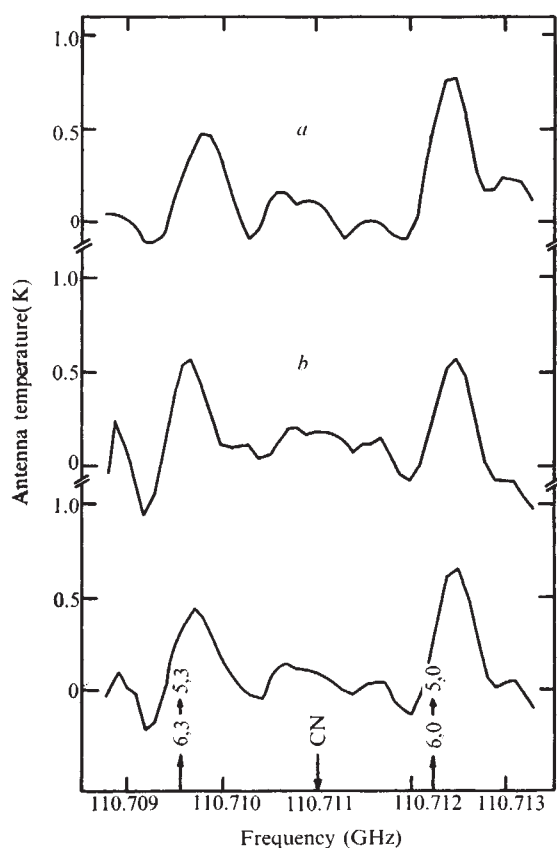


FIG. 1 Spectra of Comet Kohoutek (1973f) showing two emission lines attributed to CH₃CN: a, December 1, 1973; b, December 5, 1973; c, average.

Cosmic-ray energy spectrum from 10¹⁵ to 10¹⁸ eV

THE problem of establishing the primary cosmic-ray energy spectrum at energies $\gtrsim 10^{15}$ eV, a region of great astrophysical interest, depends for its solution on the relation between the size and total primary energy of extensive air showers. This is because the experimental data are directly related to extensive air shower size spectra, which must then be converted to a primary energy spectrum. The necessary conversion depends, among other things, on the physics of the hadronic part of the cascade and on the primary composition in an energy region in which such information is only

available from extensive air showers themselves. The primary energy spectrum above 10^{15} eV is thus less well established than it is, for example, below 10^{12} eV, where direct measurements have been made, both of cosmic-ray primaries and of individual hadronic interactions.

In the absence of independent knowledge of primary composition and of properties of high energy interactions above 10^{12} eV, a demonstration that the relation of shower size to primary energy is, to some extent, independent of these features would be valuable. Such model independence is suggested by comparison of the calculation presented here, which assumes heavy primaries and Feynman scaling to relate shower size to primary energy, with calculations based on more conventional assumptions.

Various recent calculations (refs 1-3 and other papers presented at the Denver Conference) of extensive air shower (EAS) properties show that Feynman scaling for hadronic interactions appears to break down between $\sim 1,000$ GeV and $\sim 10^6$ GeV if the primary cosmic-ray beam is predominantly protons at EAS energies ($E \gtrsim 10^6$ GeV). It has been suggested, however, that if the primaries are predominantly heavy nuclei (for example, Fe) and if intranuclear cascading and an increasing p-air cross section are taken into account, then an approximate form of Feynman scaling (modified to allow for an increasing cross section) may hold from Intersecting Storage Rings (ISR) to EAS energies (refs 1, 2 and 2a). Here I report the use of a cascade calculation based on these assumptions to calibrate the Chacaltaya measurements of size against depth⁴⁻⁶ and obtain a primary energy spectrum from 10^{15} to 10^{18} eV. The spectrum obtained in this way is surprisingly close to that obtained from entirely different assumptions by the authors of refs 4, 5 and 6. (The authors of ref. 6 point out that, in view of the disagreement (around 10^{17} eV) between their results and those of refs 4 and 5, theirs are preliminary.)

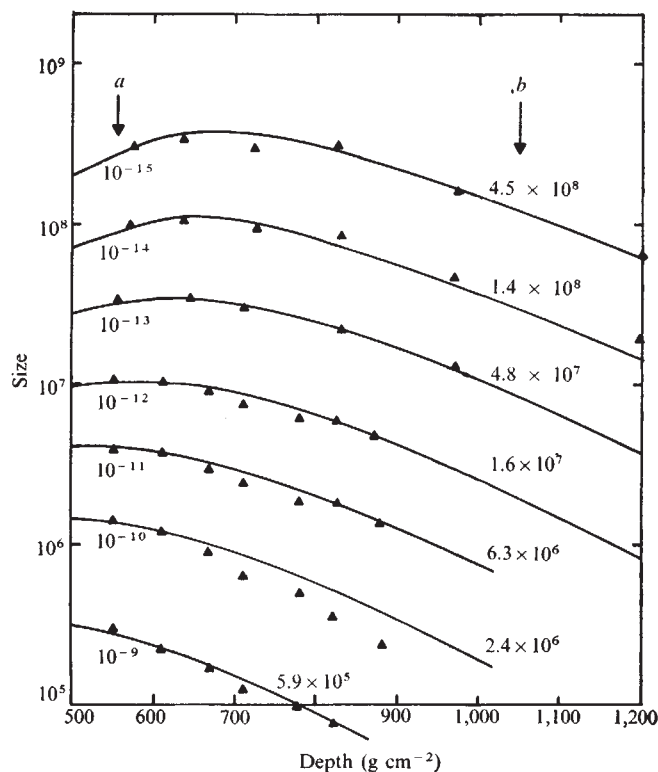


FIG. 1 Comparison between data and calculations of shower size against depth. Each calculated curve is normalised at one depth only (600 g cm^{-2}) to the data. *a*, Intensity ($\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$); *b*, corresponding total primary energy per nucleus, E_0 (GeV). ▲, refs 4 and 5.

Specifically, I assume pure iron primaries over the range of the Chacaltaya measurements, Feynman scaling⁷ from ISR data for the number distributions of pions produced in hadron-hadron interactions, and the model of Fishbane, Newmeyer and Trefil⁸ for intranuclear cascading. This picture of the primary composition is almost certainly oversimplified and possibly incorrect⁹ and the assumptions about hadronic interactions at EAS energies may have to be modified. Nevertheless, the quality of the fit to the Chacaltaya measurements of size against depth obtained in this framework makes it worth using it to convert the Chacaltaya size spectra to an energy spectrum.

In the spirit of this simple approach I treat an iron nucleus of total energy E_0 as a superposition of nucleons each of energy $E_0/56$. The size of the electromagnetic component of the shower is computed at various atmospheric depths as a function of E_0 using the cascade calculation described^{2a}.

The Chacaltaya shower development data⁴⁻⁶ were obtained by taking constant intensity cuts in the integral size spectra at various shower zenith angles, corresponding to different atmospheric depths. The minimum depth is thus the vertical depth at Chacaltaya, 550 g cm^{-2} . I obtain the energy calibration here by fitting the observed size at 600 g cm^{-2} to the calculated shower size at this depth for each intensity. The calculated shower development curves are compared with the data of refs 4 and 5 in Fig. 1. It is clear from the agreement between the curves and the data in Fig. 1 that a different choice of normalisation depth would produce

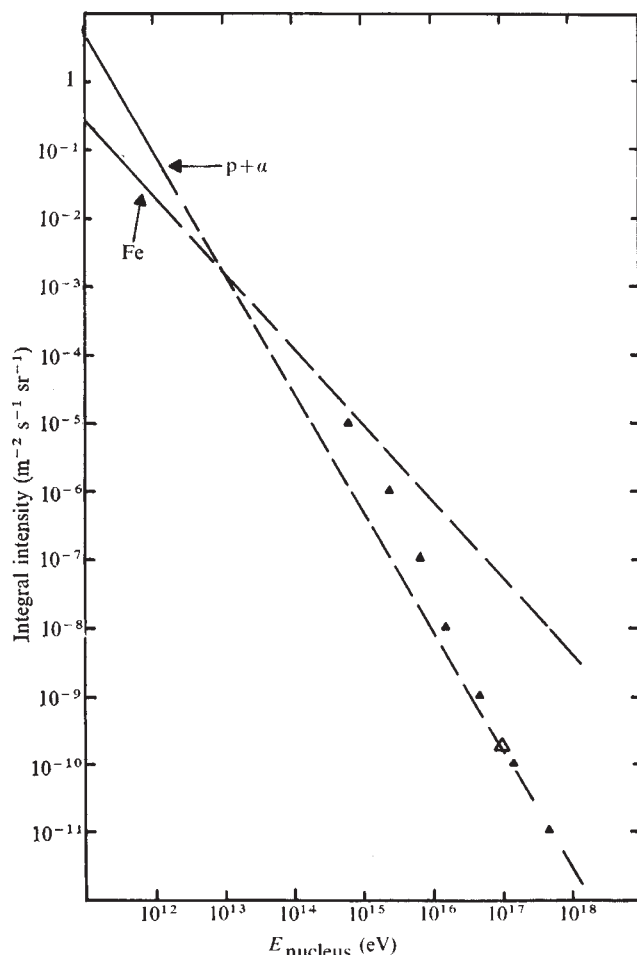


FIG. 2 Data points represent the primary integral energy spectrum obtained from Fig. 1 as described in the text. The normalisation obtained at 10^{17} eV from the EAS calibration of refs 4 and 5 is also shown as a Δ . ---, Extrapolations of Fe and $p + \alpha$ spectra measured at lower energies in balloon experiments (refs 10 and 11).

little change in the primary energy assigned to any of the intensities. Similar good agreement between calculated and observed shower development is also obtained for the data of ref. 6 (not shown here).

Figure 2 shows the integral energy spectrum of EAS primaries obtained from this analysis. A similar analysis has been carried out for the data of ref. 6. Both the magnitude and the slopes obtained agree within errors with those determined in refs 4-6 assuming predominantly proton primaries and using constant cross section and power law multiplicity for the number of particles produced in hadron-hadron collisions. In particular, the slope of the primary spectrum is steeper above 10^{15} eV than at lower energies ($\gamma \sim 2.2$ for the data of refs 4 and 5 and $\gamma \sim 1.8$ above 10^{17} eV for ref. 6). The similarity of primary energy spectra obtained under these two extreme sets of assumptions gives one confidence that the energy spectrum is not subject to appreciable uncertainties arising from ignorance of primary composition or of hadronic production mechanisms in this energy range (provided one uses a picture that fits the data on size against depth for EAS).

For comparison I show in Fig. 2 the data of the Goddard Space Flight Center on iron¹⁰ (plotted as number of Fe with total energy $>E$) and $p + \alpha$ primaries¹¹ (solid lines) and their extrapolations (dashed lines). Clearly, if the primary spectrum above $\sim 10^{15}$ eV is all iron, the slope of that spectrum is unlikely to have any relation to the mechanism¹² that may be responsible for the flat iron spectrum at low energy.

I thank G. B. Yodh for discussions and P. K. MacKeown for making available the results of ref. 6. This work was supported in part by the National Science Foundation.

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Raman backscatter of Laser radiation from the stratosphere

RAMAN-shifted backscatter of laser radar radiation from atmospheric constituents in the troposphere has been reported¹⁻³. Signals from nitrogen have been detected up to heights of about 6 km and there have been indications of backscatter at even greater heights⁴. Oxygen and water vapour have also been the subject of tropospheric Raman investigations, generally using frequency doubled ruby (0.3472 μ m) as the laser transmitter.

Results outlined below show that the Mark II laser radar

system at the University of the West Indies, Kingston, Jamaica, can extend the range of Raman investigations on atmospheric nitrogen well into the stratosphere. This system has been described⁵ and is unchanged except for the introduction of new digital counting circuitry. A pulsed ruby laser transmits at a wavelength of 0.6943 μ m and the Raman return from nitrogen is received at 0.8284 μ m. The returning radiation passes through an interference filter (bandwidth 1.5 nm) on to photomultipliers and then, by way of a suitable pulse discriminating and amplifying system, to counters which give a digital display of the photon count for gated 2 km and 4 km intervals. Although there is some disadvantage in scattering cross section and photomultiplier sensitivity at these wavelengths, data obtained indicate that signals can be detected up to a height of about 45 km.

In order to examine statistically the performance of the system, the photon counts from the different gates have been related to atmospheric density values obtained from balloon measurements carried out by the local Meteorological Office and from the US Standard Atmosphere Supplements for 15° N⁶. The laser radar site is at Kingston (18.0° N) and is approximately 10 miles from the point at which the Meteorological Office sends up balloons. Comparisons have had to be made with the US Standard Atmosphere as the balloons used by the Meteorological Office burst in the region of 30 km. In addition, the accuracy of balloon measurements rapidly diminishes above 20 km.

The received photon count C is expected to be proportional to the ratio $(\rho_0 T_v T_D / h^2)$ where ρ_0 is the atmospheric density; T_v the atmospheric transmissivity at 0.6943 μ m, T_D the atmospheric transmissivity at 0.8284 μ m, and h the distance from transmitter to the effective point of scatter. As it is not possible to determine directly the constant of proportionality, the ratio $(Ch^2 / \rho_0 T_v T_D)$ has been normalised to unity at the gate interval 22-24 km. (This point was chosen for technical reasons associated with the equipment and photon counting method.) For perfect correspondence of C and ρ_0 , the ratios for all other gates should be unity within the limits of probable error. Figure 1a shows the photon counts obtained from an investigation on the night of August 25, 1973, when 2,200 laser firings were made, compared with atmospheric density values obtained from the US Standard Atmosphere Supplement (annual average).

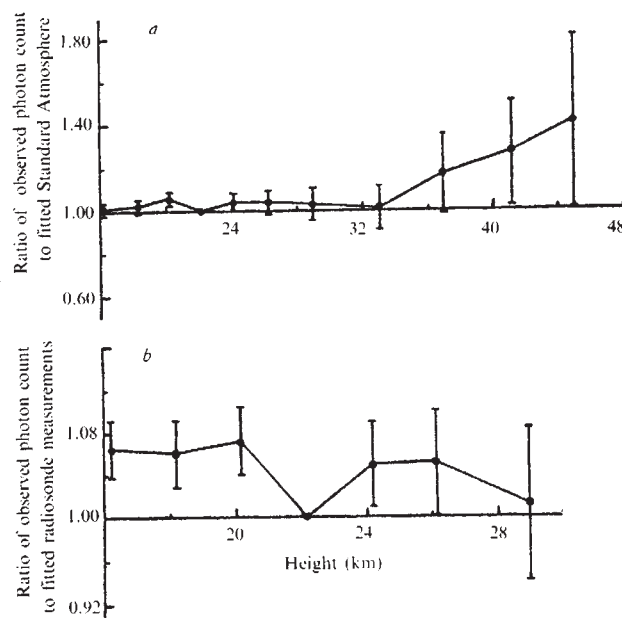


FIG. 1 Photon counts obtained on the night of August 25, 1973, compared to (a) US Standard Atmosphere Supplement for 15° N; (b) local radio-sonde measurements. The observations have been fitted at a height of 22 km.

This comparison is appropriate since the atmosphere at this latitude is remarkably constant. Figure *b* shows the same data related to radiosonde measurements made by the Jamaican Meteorological Office at 0700 h EST on August 26, 1973. In estimating the performance of the laser radar system, it must be noted that at a height of 28 km the pressure is in the region of 15 mbar, and radiosonde measurements of pressure can be in error by as much as 15%. There are also error contributions due to temperature and height measurements.

There is close agreement between the laser radar measurements and data from the Meteorological Office and the US Standard Atmosphere Supplement. The error bars show the standard deviation of the laser radar measurements based on the counting statistics. Most points are within one standard deviation of unity and none are much more than two distant from it. No allowance has been made for any possible errors in the radiosonde data. We think the results confirm that useful Raman signals from atmospheric nitrogen can be detected by the Mark II system up to a height of about 40 km. In the final gate (44–48 km) the signal-to-noise ratio was approximately unity whereas for the 36–40 km gate it was greater than 3.

We thank the Jamaica Meteorological Service for making radiosonde data available. Operation of the Mark II laser radar system has been partially financed by the Air Force Cambridge Research Laboratories, Office of Aerospace Research, United States Air Force.

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TABLE 1 Field data used to calculate K_x and U

Survey		Southern boundary	Northern boundary	Caesium in channel (Ci)
May 1972	S	34.63	34.30	4,073
	C_s	1.8	8.7	
	$\frac{\partial C_s}{\partial x}$	0.04	0.1	
September 1972 RRS John Murray Cruise 11-72	S	34.75	34.25	3,802
	C_s	1.3	7.2	
	$\frac{\partial C_s}{\partial x}$	0.03	0.13	
February 1973 MV Researcher Cruise 02-73	S	34.77	34.36	3,634
	C_s	1.7	12.0	
	$\frac{\partial C_s}{\partial x}$	0.01	0.19	
June 1973 RRS John Murray Cruise 05-73	S	34.63	34.30	5,018
	C_s	1.7	16.0	
	$\frac{\partial C_s}{\partial x}$	0.01	0.21	

Salinities from Bowden⁴. Caesium values (May 1972) from Jefferies *et al.*⁶; others from unpublished data of this laboratory. Units: Salinity (S) parts per thousand by weight; ^{137}Cs pCi⁻¹ (equivalent to Ci km⁻³).

transport of salt by turbulent mixing the results he obtained (400 to 700 km yr⁻¹) are rather higher than those obtained by methods which take account of this process². Proudman³ correlated the passage of salinity anomalies at various observation points in the Irish Sea system and obtained a travel time corresponding to about 400 km yr⁻¹; Bowden⁴ utilised improved data to obtain a value of about 150 km yr⁻¹ by a similar method. Proudman⁵ derived an estimate for the ratio of the flow velocity to the diffusion coefficient normal to the flow direction by fitting a parabola to the observed isohalines between Holyhead and Dublin; this approach gave estimates of between 750 and 270 km yr⁻¹ depending on the value assumed² for the diffusion coefficient.

Bowden² extended Knudsen's method by including diffusive terms in the equation of continuity of salt. The continuity equations he obtained are as follows.

For water

$$X_1 U_1 + W = X_2 U_2 \quad (1)$$

(where X_1 and X_2 are the south and north boundary cross-sectional areas respectively (Fig. 1), U_1 and U_2 are the corresponding mean advective velocities (northwards positive) and W is the net fresh water input to the channel from precipitation, evaporation and runoff).

For salt

$$\begin{aligned} \rho_1 X_1 U_1 S_1 - \rho_1 X_1 \left(K_x \frac{\partial S}{\partial x} \right)_1 \\ = \rho_2 X_2 U_2 S_2 - \rho_2 X_2 \left(K_x \frac{\partial S}{\partial x} \right)_2 \end{aligned} \quad (2)$$

where ρ and S represent densities and salinities respectively and the second term on each side of the equation represents the mass flux of salt through the appropriate cross section by turbulent mixing. This equation may be simplified by assuming that $\rho_1 = \rho_2$ and K_x is constant: these are reasonable assumptions for the St George's Channel, where the north and south cross sections are similar.

Because K_x , U_1 and U_2 are unknown it is not possible to obtain a direct solution by considering only equations 1 and 2. To circumvent this problem Bowden² assumed K_x at the southern boundary to lie between 0 and $8.8 \times 10^6 \text{ cm}^2 \text{ s}^{-1}$. Using this assumption he estimated that U_1 lay between 126 and 610 km yr⁻¹ and U_2 between 111 and 438 km yr⁻¹.

Study of the movement of ^{137}Cs , released into the Irish Sea as a component of the low level radioactive waste pro-

Caesium-137 as a water movement tracer in the St George's Channel

CALCULATIONS based on recent measurements of the distribution of ^{137}Cs in the St George's Channel show that the residual (non-tidal) flow is slightly lower than previous indirect estimates suggested. The contribution of turbulent mixing to the transport of dissolved and particulate material in these waters may also be calculated directly from caesium measurements.

Study of the isohaline patterns and of drifter movements shows that there is a residual flow of water from south to north in the Irish Sea system. It is, however, difficult to arrive at an accurate mean value for this flow because of the effects of turbulent mixing in modifying the isohaline pattern, and the effects of wind on surface drifters. Available current meter measurements and subsurface drifter experiments are insufficient in number to give long term mean values for such a large scale low velocity flow.

Knudsen¹ approached the problem by application of the continuity conditions for water and salt. Since he neglected the

duced at the Windscale plant of British Nuclear Fuels Ltd, provides a means to the direct solution of this problem. Caesium-137 is an almost ideal tracer for seawater movement as it has a long half-life (~ 30 yr), does not adsorb significantly on to sedimentary material and is easily detected at very low concentrations. Since the amounts of tracer added are several orders of magnitude less than the seawater concentration of natural (non-radioactive) caesium ($0.3 \mu\text{g l}^{-1}$), the presence of the tracer has no influence on the density or other physical properties of the seawater. It is, however, necessary to separate the tracer from the natural background activity of seawater due to ^{40}K (200 pCi l^{-1}) and to concentrate it for counting. In the present work this is done by adsorbing the tracer from up to 10 l of seawater onto a small volume (1 ml) of copper ferrocyanide. The retention of caesium is essentially complete (98%) whereas potassium is not adsorbed. Data for three complete surveys of the Irish Sea system have been obtained by this method (Table 1); the results for February 1973 are shown in Fig. 1. The results

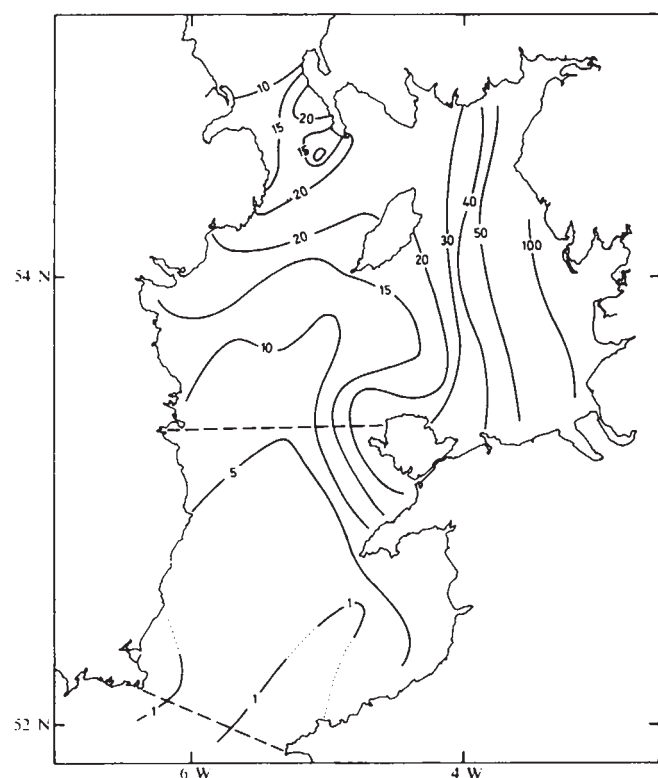


FIG. 1 Distribution of caesium-137 in the St George's Channel and Irish Sea during February 1973. Survey made on ICOT cruise, RS-02-73. Units are pCi l^{-1} . North and south boundaries of the St George's Channel are shown by dashed lines.

of an additional survey⁶ made during May 1972 have also been used in the present study. These analyses were carried out by a method broadly similar to that outlined⁷.

Since the concentrations of ^{137}Cs in the St George's Channel are not at steady state the continuity equation takes the following form

$$X_1 U_1 C_{s1} - X_1 K_x \left(\frac{\partial C_s}{\partial x} \right)_1 = X_2 U_2 C_{s2} - X_2 K_x \left(\frac{\partial C_s}{\partial x} \right)_2 + P \quad (3)$$

where P is the increase in ^{137}Cs within the channel volume during the time period under consideration. This quantity

may be obtained by integration of the ^{137}Cs values, shown in the survey contour charts, over the whole water volume. For this purpose the channel was divided into rectangles $10'$ latitude by $30'$ longitude (604 km^2); each was assigned an equivalent volume based on the mean depth and the fraction of the total area covered by the water. Measurements of the depth distribution of caesium⁸ show that in this region the concentration of caesium does not vary significantly with depth. Consequently in this study the surface value was assumed to apply to the whole water column.

Equations (1), (2) and (3) may be solved for the three unknowns

$$K_x = \left\{ W S_2 C_{s1} - W S_1 C_{s2} + P(S_2 - S_1) \right\} \left\{ X_2 \left(\frac{\partial C_s}{\partial x} \right)_2 - X_1 \left(\frac{\partial C_s}{\partial x} \right)_1 (S_2 - S_1) - X_2 \left(\frac{\partial S}{\partial x} \right)_2 - X_1 \left(\frac{\partial S}{\partial x} \right)_1 (C_{s2} - C_{s1}) \right\}^{-1} \quad (4)$$

$$U_1 = \left\{ K_x \left(X_2 \left(\frac{\partial S}{\partial x} \right)_2 - X_1 \left(\frac{\partial S}{\partial x} \right)_1 \right) - W S_2 \right\} \cdot \{ X_1 (S_2 - S_1) \}^{-1} \quad (5)$$

$$U_2 = \{ X_1 U_1 + W \} X_2^{-1} \quad (6)$$

These equations may be evaluated numerically for the time period between each survey using the values given in Table 1 and the information on channel geometry and freshwater flow given in ref. 2. The results, which are given in Table 2, show excellent internal consistency and fall close to the range of values estimated by Bowden.

TABLE 2 Summary of results

Period	K_x $\text{km}^2 \text{ yr}^{-1}$	10^6 cm^2 s^{-1}	U_1 km yr^{-1}	U_2 km yr^{-1}
May to September 1972	8,130	2.59	129	113
September 1972 to February 1973	8,160	2.60	116	103
February to June 1973	8,980	2.86	141	124
Surveys before 1972				
Mean	11,600	3.7	97	88
Range	9,500-16,000	2.7-5.1	103-88	91-78

Bowden also considered other sections of the Irish Sea system to the north and south of the present area of study. Attempts to apply the continuity equation for caesium to the most northerly section (the Irish Sea proper) have shown that the assumption of constant K_x leads to anomalous results for this section. This is not surprising in view of the intense and complex mixing and advective processes in the North Channel (see Fig. 1) and the obvious dissimilarities between the north and south boundaries of this section. A further programme of study in this area is being undertaken. South of the St George's Channel (Celtic Sea) the radiocaesium levels are too low for reliable application of the present approach.

It is interesting that the total quantity of ^{137}Cs in the St George's Channel reflects the fact that about one third of the total ^{137}Cs input to the Irish Sea during 1972 was made in the August of that year (H. Howells, personal com-

munication). This 'pulse' apparently arrived at the northern boundary of the St George's Channel at about the time of the survey in February 1973 and shows as a sudden increase in total ^{137}Cs in the results for June 1973. The travel time of 8 to 10 months for passage through the Liverpool Bay system gives an apparent velocity of about 200 km yr^{-1} ; it is possible, however, that the mechanism of transport is predominantly diffusive rather than advective, since tidal mixing is very strong in this region.

In addition to the data used in the above calculations three other, less complete surveys are relevant. These were made as part of the research programme of the Ministry of Agriculture, Fisheries and Food Fisheries Radiobiological Laboratory, Lowestoft. The survey dates are September 1968, December 1969 (ref. 8) and June 1970 (D. F. Jefferies, personal communication). Although none of these surveys includes information on the southern boundary of the St George's Channel it is possible to make estimates of the values in this area from known North Atlantic fallout levels (1.0 to 0.3 pCi l^{-1}) and from the later surveys. This procedure permits the estimation of residual advective velocities and diffusion coefficients for four additional periods between surveys. The range of values of K , so estimated is given in Table 2. The bias to slightly higher values shown by these estimates when compared with the calculated results may reflect the longer time periods involved, because advective processes operating for periods short relative to the time period concerned cannot be distinguished from diffusive processes and thus tend to increase the apparent diffusion coefficient.

The presence of ^{137}Cs in the Irish Sea system presents a unique opportunity to study large scale advective and diffusive processes over long time and distance scales. The present paper and that of Jefferies *et al.*⁶ illustrate only a few of the ways in which this fortuitous situation can be exploited to improve our knowledge. The near-shore transport processes here investigated control the movement and speed of all natural or artificial materials introduced into the waters around our coasts.

I thank the many people who assisted me with the sampling programme on board RRS John Murray and MV Researcher, and Dr J. P. Riley of the University of Liverpool for the loan of laboratory facilities. Mr H. Howells of British Nuclear Fuels Ltd, Windscale, provided information on the input of ^{137}Cs to the Irish Sea.

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An additional location of metalliferous sediments in the Red Sea

INTEREST in Red Sea brines and metalliferous sediments commenced in 1948 with reports of anomalously high bottom water temperatures in the vicinity of Atlantis II Deep¹. These reports were confirmed in the mid 1960s (refs 2-4) and subsequent investigations have revealed at least thirteen occurrences of brine pools and metalliferous sediment throughout the length of the Red Sea⁵⁻⁷. Detailed sampling of one deep, Nereus Deep ($23^{\circ} 10' \text{N}$, $37^{\circ} 15' \text{E}$), has indicated the presence of brines and metalliferous sediments of distinctive composition.

Nereus Deep is situated within the NW-SE trending median valley of the Red Sea, is 40 km long by 12 km wide, and is divided along its length by a saddle into two parallel basins (Fig. 1). Each basin contains a number of distinct depressions which either contain brine pools, or show evidence of having contained them in the past. The largest brine pool occurs in the deeper eastern basin filling two of the depressions, which appear to be separated by a low sill (Fig. 1). This brine has a chlorinity of 129.5‰ and a temperature of 30.2°C (ref. 7). In the western basin there is a smaller brine pool, which has a lower salinity, and may be present as a re-

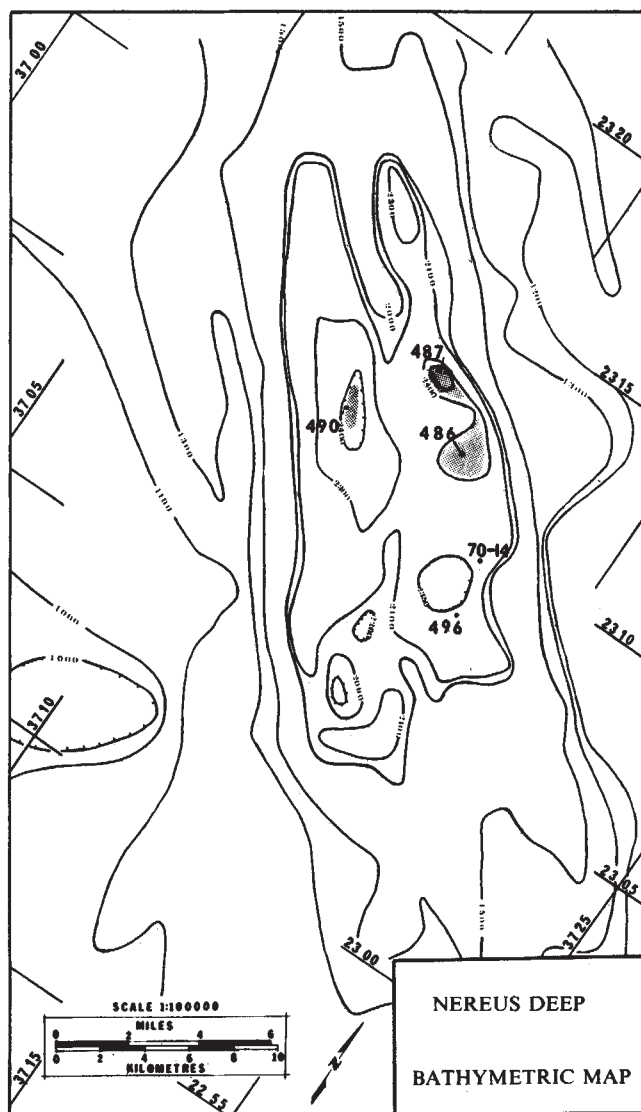


FIG. 1 Bathymetric map of Nereus Deep. Depths of water are in m.

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TABLE 1 Average composition of metalliferous sediment layers from Nereus Deep

	70-14 8-9 cm	70-14 12-27 cm	70-14 30-35 cm	70-14 54-108 cm	496 0-320 cm	496 320-470 cm	486 0-48 cm	486 115-123 cm	486 190-200 cm	486 220-236 cm	487 0-54 cm	487 115-150 cm	487 238-257 cm	487 321-354 cm	490 0-33 cm
Fe%	29.70	26.80	20.10	29.40	20.74	40.35	6.70	2.70	17.00	3.00	4.80	10.90	12.55	5.20	5.83
Mn%	16.40	15.10	18.00	17.30	11.94	0.38	3.80	0.35	1.80	0.50	1.10	1.15	2.08	1.30	1.53
Zn p.p.m.	10,900	11,010	10,200	11,115	9,400	4,820	2,400	450	382	350	904	590	410	700	784
Cu p.p.m.	835	458	167	201	332	41	48	150	365	70	72	280	402	85	87
Ni p.p.m.	6	12	25	7	15		12	25	21	60	12	7	24	20	33
Co p.p.m.	15	12	15	12	14	8	16	30	30	30	16	10	32	25	39
Cr p.p.m.	20	15	15	15	42	33	14	20	18	12	19	12	25	20	44
Pb p.p.m.	370	380	200	495	314	540	42	50	20	40	92	53	64	70	60
* Hg parts per 10 ⁹	390	420	620	748	451	96	108	70	80	72	105	68	90	80	45
Ca %	4.20	5.60	6.00	3.80	2.88	0.88	10.20	18.00	7.00	18.00	11.50	12.00	10.70	15.00	17.00
† Salt %	15.0	14.5	14.0	14.5	14.5	18.5	48.0	30.0	47.0	23.0	47.0	48.0	41.0	30.0	14.3

* Core 70-14 was stored for 2 yr before analysis for Hg.

† Weight loss on washing.

sult of seepage or spillage from the main pool in the eastern basin.

Geochemical analysis of unwashed samples of sediment cores from the Deep has been undertaken for Fe, Mn, Zn, Cu, Pb, Ni, Co, Cr, Hg and Ca using atomic absorption techniques. This has confirmed the observed presence of several metalliferous layers within the sediments.

The most highly metalliferous sediments within the Deep occur in core 70-14 from the south-east slope (Fig. 1) and are apparently unrelated to any existing brine. This core contains four distinct layers of fine grained black-brown sediment of high water content. The true thickness of the deepest of these layers was not determined as the corer stopped within it, but 50 cm of metalliferous material was recovered and the possibility that they formed as a result of the oxidation of these black layers (Table 1) shows Fe to be very enriched, with Mn, Zn, Cu, Pb and Hg also enriched. These layers are chemically most similar to the 'manganite' facies of Chain Deep⁸. Core 496 (Fig. 1) taken from a greater depth in the south-east corner of the deep also contains 3.2 m of iron and manganese rich sediment (Table 1). Below this is at least 1.5 m of Fe-rich sediment.

The origin of the black manganese rich sediments is difficult to explain owing to the absence of any direct connection with existing brine. Possibly they were laid down in association with pre-existing brines, or result from diagenetic remobilization of previously buried manganese dioxide under reducing conditions. Perhaps more likely, however, is the possibilities that they formed as a result of the oxidation of dissolved manganese at the brine/seawater interface, with subsequent precipitation of manganese dioxide around and above the margins of the brine pool (H. Bäcker, personal communication).

Cores such as 486 and 487 from beneath the brine pools in the eastern basin (Fig. 1) show at least four distinct periods of brine influx (Table 1) during which fine banded metal-rich layers were deposited. Examination of the micro-fossil assemblages of these two cores has shown that the four metalliferous layers can be correlated (S. Ali, personal communication). In core 486 two of these layers are thinner and less metalliferous than the corresponding sediments in core 487, suggesting the sill between these two depressions may have inhibited brine flow from north to south. The brine precipitates have a high salt content and are enriched in Fe, Mn, Zn and Cu (Table 1).

The western basin seems to have had a much shorter history of brine deposition than the eastern basin, brine precipitates only being found in the upper 50 cm of the sediment column below the present brine pool. The salt and metal contents of these precipitates are much lower than in the eastern basin (Table 1), suggesting that the brines in the western basin may have been mixed with normal Red Sea water. The level of initial brine influx coincides with a zinc

and manganese rich layer, containing the maximum values of these elements in core 490 (Table 1).

Major points of interest in Nereus Deep are, therefore, first the occurrence of an appreciable thickness of iron and manganese-rich sediment containing various minor elements and, second, the possibility that, as in Atlantis II Deep^{8,9} sulphide layers rich in zinc, copper and lead may occur at greater depths. Large concentrations of mercury in the Deep (Table 1) support this view and may indicate hydrothermal enrichments of less volatile elements below.

This work was supported by a grant from the Natural Environment Research Council. Ship time was provided by the Directorate General Mineral Resources, Saudi Arabia. We also thank Dr H. Bäcker of Pressaug AG for providing sediment samples and a detailed bathymetric map of the deep.

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Crystal formation and growth in bivalve nacre

THERE are two theoretical explanations of the initiation and growth of the tabular aragonite crystals forming the nacreous layer in molluscan shells. The 'template' or 'matrix' hypothesis assumes that crystal nucleation and growth occur on the open surface of conchiolin membranes and may depend on active sites of this organic substratum². This implies a process comparable to epitaxy. In contrast, the 'compartment' hypothesis envisages that nucleation and growth occur within, and are controlled by, pre-existing hollow compartments of the organic deposits³. Two recent

scanning electron microscope (SEM) studies⁴ indicate that in gastropods there are no such compartments at the growth front of the nacreous layer. These studies are, however, not completely conclusive because of insufficient fixation of the samples. We have therefore studied the formation and growth of initial nacreous crystals in several species of bivalves.

We selected young individuals of *Pteria colymbus*, *Isognomon alatus*, *I. radiatus*, *I. bicolor* and *Brachidontes exustus* in which active shell growth was in progress. The molluscs were put in 6% solution of magnesium sulphate in seawater until the tissues were relaxed, then fixed by a 2% solution of glutaraldehyde in cacodylate buffer prepared in seawater for 4 h at room temperature.

After rinsing in buffer, the desired portions of the shells were cut off with the mantles attached. For transmission electron microscopy (TEM), they were dehydrated in an ethanol series, embedded in Spurr⁵ plastics, and sectioned with a diamond knife. Sections were stained with Reynold's lead citrate⁶ and/or uranyl acetate. The uranyl stain also served for decalcification.

For SEM, shells were dehydrated using the critical point method and the mantles pulled off to expose the inner shell surfaces; the samples were then coated with gold-palladium under vacuum.

Observations with the SEM confirmed that in contrast to gastropods^{4,7} the nacreous layers of these bivalves are formed in a step-like pattern (Fig. 1a), each step corresponding to the growth edge of a nacreous lamina. In front of each lamina appear widely scattered crystals which are in the initial stage of formation. They grow by fast lateral and slower vertical accretion, acquiring first discoidal or sub-hexagonal habits and later fusing into single groups which finally merge to form a coherent nacreous lamina.

Transmission electron microscopy observations of the sections of mantle/shell preparations from semi-mature laminae (laminae crystals of which have not completely fused) reveal a situation similar to that described by Bevelander and Nakahara in other bivalve species³. Gaps are often found

between some of the crystals or crystal groups of the latest nacreous lamina. Some of the gaps contain traces of poorly condensed shred-like organic material (Fig. 1b, c). In addition, one or more sheet-like formations of condensed organic material have been observed between the microvilli of the mantle epithelium and the inner face of the nacreous layer. The innermost sheet may correspond to the mucous lining of the microvilli and the other formations may be independent membranous sheets. Examination of larger parts of the inner shell surface with the SEM has shown that such sheets extend over several nacreous steps (Fig. 1a). Thus, in the initial parts of the nacreous laminae these sheets are not strictly parallel to the surfaces of the laminae.

Near the edge of the shell is an organic lining which occupies the junction between the prismatic and the nacreous layer. It extends adorally beyond the growth front of the nacreous layer and covers part of the inner face of the prismatic layer.

The earliest initial stages of nacreous crystals appear on the surface of this lining. In some cases this occurs preferentially in the furrows representing the borders of the underlying prisms (Fig. 1d). A short distance behind they become fused to form the first coherent aragonitic lamina. In some cases the membranous sheets reach far enough forward to cover the growth front of the nacreous layer or even the inner face of the prismatic layer (Fig. 1d).

Our observations have shown that 'compartments' in the functional sense attributed to this term do not exist. In the semi-mature nacreous lamina the gaps covered by the membranous sheet are neither spaces controlling crystal formation and growth nor the result of crystal dissolution⁴ but simply places where the lateral fusion of the nacreous crystals has not been completed. They are certainly not compartments in the sense of the compartment hypothesis. On the other hand, the spaces between two adjacent horizontal membranous sheets as observed by Bevelander and Nakahara could be called compartments if the meaning of this term is restricted to a purely descriptive sense. But it seems questionable whether they control crystal growth.

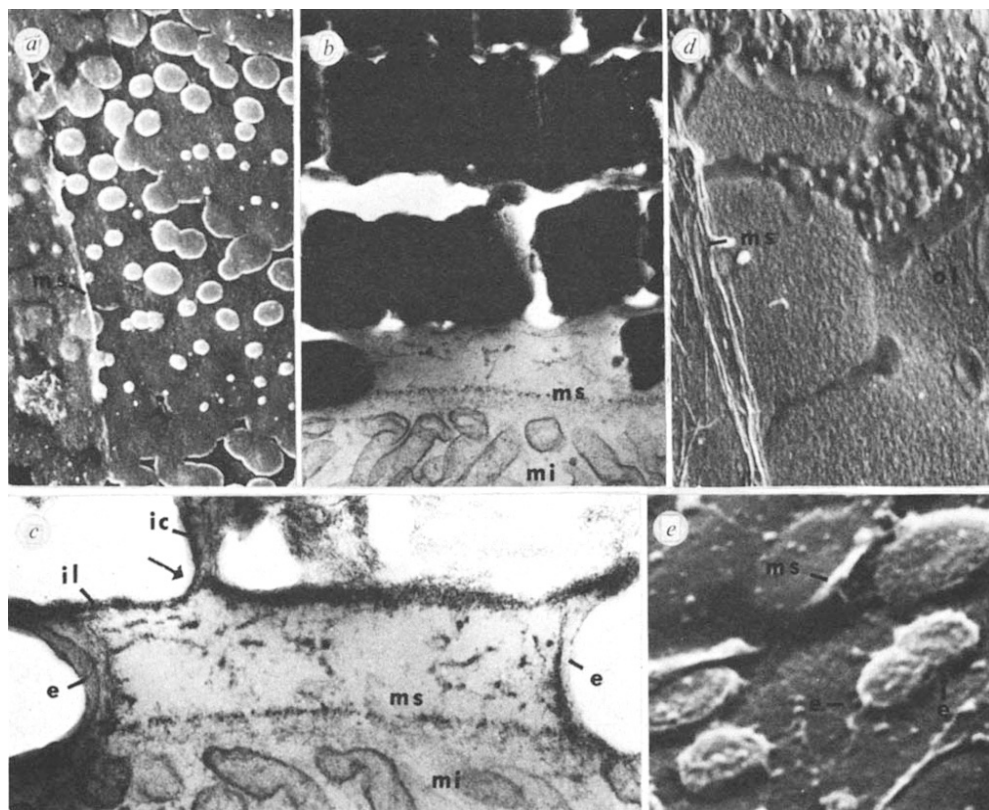


Fig. 1 Transmission and scanning electron micrographs of growing nacre in bivalves. a, Growing edges of three consecutive nacreous laminae in *Pteria colymbus*, all covered by a membranous sheet removed at the right side. Vertical view. $\times 1,680$. b, Gap in an incompletely fused lower-most nacreous lamina of *P. colymbus*. Vertical section. $\times 35,952$. c, Same. Note the organic envelopes of the crystals and the junction in the intercrystalline conchiolin membrane (arrow). Vertical section, decalcified. $\times 52,248$. d, Growth front of the nacreous layer (upper parts) covering the prismatic layer (lower parts) in *Isognomon alatus*. Note the organic lining underlying the nacre and the membranous sheet covering both layers and removed at right sides. Vertical view. $\times 336$. e, Surface of a growing nacreous lamina in *P. colymbus*. Note the organic envelopes of the crystals and the covering membranous sheet removed in the lower parts. Oblique view. $\times 6,720$. e, organic envelope of nacreous crystals; ic, intercrystalline conchiolin membrane; il, interlamellar conchiolin membrane; mi, microvilli; ms, membranous sheet; ol, organic lining underlying the nacreous layer.

It is true that they contain shreds of organic material in the initial state of consolidation and may, therefore, represent a separate micro-environment enclosing a modified pallial fluid³. Crystal formation and growth, however, seem to be rather independent as each of the initial nacreous crystals has its own organic envelope (e in Fig. 1c) which is independent of the organic sheets of the compartment (Fig. 1e). It is also inconceivable that the invariant vertical distance of the membranous sheet from the surface of the preceding nacreous lamina should be responsible for the uniform thickness of the newly formed mature crystals or lamina. Since this sheet is not consistently parallel to such a surface, its distance is not constant. We, therefore, conclude that the uniform thickness of the nacreous laminae cannot be attributed to the membranous sheet.

According to our observations initial and growing nacreous crystals are covered by a rather "tightly fitting organic envelope"⁴ consisting of one or several very fine linings (Fig. 1c, e) which are somewhat more conspicuous near the base of the crystal (Fig. 1e). During the lateral fusion of the crystals to form a mature nacreous lamina the adjacent lateral portions of the envelopes obviously merge (Fig. 1c) to become Gregoire's "intercrystalline conchiolin membrane". Such fusion of parallel organic membranes of identical structure and composition leaves no traces of a separating boundary. In our case, however, it is implied by the occasional preservation of their junction (arrow in Fig. 1c). The lateral fusion of the upper portions of the crystal envelopes results in the formation of a horizontal organic lamellar complex in which the original separations may remain detectable (arrow in Fig. 1c). This complex and the inner membranous sheet of the compartment forms Gregoire's "interlamellar conchiolin membrane" (see ref. 8).

The tightly fitting envelope of the growing crystals could easily be mistaken for a single membranous sheet belonging to the compartment and draped over the crystals following a collapse of the compartment during the preparation. Such an interpretation is, however, unlikely because the samples have undergone proper fixation and critical point dehydration. Finally it is invalidated by the observation that the envelope extends far under the latero-basal parts of the crystal (Fig. 1c).

There is, of course, the question of how this envelope is able to increase its surface as the crystal within it grows. It is, however, not a rigid structure. Since conchiolin membranes of the nacreous layer represent a network of tiny fibrils (see ref. 8) they can easily be stretched while new fibrils are added to fill the opening meshes. A good model is the avian eggshell membrane which is stretched as the egg grows in the isthmus.

Another question is why the organic envelope does not actually inhibit further growth of the crystal. There is still no answer to this but further investigation may show perforations or permeability of this membrane. Finally, why does the vertical growth of adjacent crystals cease at exactly the same level so that the mineral lamina resulting from their lateral fusion is uniform in thickness?

The evidence presented here shows that the formation and growth of nacreous crystals occur independently of the existence of the compartments from which these crystals are separated by individual tightly fitting organic envelopes. Since this envelope does not delimit a pre-existing space it is not a compartment in the sense of the compartment hypothesis. According to our observations the crystals at the growth front of the nacreous layer are formed on the surface of the organic lining which advances over the inner face of the prismatic layer. At the growth edges of subsequent nacreous laminae such initial crystals are formed on the surface of the interlamellar conchiolin membrane of the preceding nacreous lamina. Thus in both cases crystal nucleation occurs on the surface of an organic substratum,

an observation compatible with the template hypothesis.

This work was supported by a US Public Health Service grant from the National Institute of Dental Research. It was done during a visit by H.K.E. to the Electron Microscope Laboratory and the Department of Geology, University of South Carolina, Columbia, South Carolina. We thank Dana Dunkelberger for technical assistance, James D. Thomas for help in securing the molluscan material and Professor Winoma Vernberg for help in editing the manuscript.

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Possible cosmic dust origin of terrestrial plutonium-244

THE discovery of ²⁴⁴Pu by Hoffman *et al.*¹ in bastnaesite, a Precambrian rare earth fluorocarbonate ore from the Mountain Pass deposit in California, has raised the question of whether the so-called extinct nuclide, with a half life of 82 m.y., is part of the extant (or almost extinct) nuclide group or whether it was brought to Earth from a cosmic-ray source before the formation of the ore^{1,2}. I have reinvestigated the implications of its present existence in nature in terms of three possibilities: (1) survival of the primaevial ²⁴⁴Pu, (2) influx as a heavy cosmic-ray component, and (3) inflow as a cosmic dust component from supernova remnants. The result shows that there are some difficulties with the first two possibilities, both of which were thought to be plausible at the time of the discovery. Although it is still a speculative proposition at present that any part of the cosmic dust falling on the Earth originated outside of the Solar System, a calculation suggests that the ²⁴⁴Pu may have been carried by such dust. The ¹²⁹Xe excess detected by Srinivasan *et al.*³ in an old Australian iodyrite, AgI, is also not inconsistent with the present hypothesis.

The observed amount of ²⁴⁴Pu, 2.0×10^7 atoms, in the 8.5 kg of ore reported in ref. 1 would correspond to an average terrestrial abundance of 2.0×10^{-24} g g⁻¹ or an average crustal abundance of 1.0×10^{-22} g g⁻¹, if the enrichment factor of plutonium was the same as that of cerium during the formation of this ore. The cerium enrichment factor is stated in ref. 1 to be a factor of the order of 5×10^5 greater than its average terrestrial abundance, or about 10^4 greater than its abundance in the Earth's crust. The former value for plutonium concentrations is consistent with an upper limit of 3×10^{-22} g g⁻¹ for the average terrestrial value obtained

earlier by Fields *et al.* from a 1.6 kg gadolinite from the Iveland district of southern Norway⁴.

An amount of 7.8×10^{-27} g ^{244}Pu g $^{-1}$ is expected for the average terrestrial abundance from the primaevial meteoritic abundance of $[^{244}\text{Pu}/^{238}\text{U}] = 0.013$ (ref. 5) and $[\text{U}] = 2.0 \times 10^{-2}$ p.p.m. (ref. 6 and references cited therein). This expected amount is such that the chemical enrichment of plutonium would have to be about 260 times more than that of cerium, as discussed in a similar way by the original investigators¹. The requirement of this high enrichment of plutonium (1.3×10^3) relative to the cerium enrichment (5×10^5) seems to be difficult to accept in terms of the possibility of a primaevial origin in view of the known chemical similarities between the two elements. Alternatively, the observed concentration may reflect a higher initial abundance of ^{244}Pu at the time of the Earth's formation (4.56×10^9 yr ago) relative to the initial chondritic abundance. A solution to the argument must await a substantiation of the discrepancy by further studies on other samples of bastnaesite.

Price *et al.*⁷ summarised the heavy cosmic-ray track data obtained by balloon flights up to 1971 to be two tracks due to $Z = 96 \pm 2$ per $2,110 \text{ m}^2 \text{ h}$. It may be that an order of one track per $2,110 \text{ m}^2 \text{ h}$ is recorded by ^{244}Pu , which leads to a rate of influx of 5×10^{-33} g ^{244}Pu cm $^{-2}$ s $^{-1}$. Assuming a steady state cosmic-ray source and a factor of survival against nuclear disintegration during passage through the atmosphere of 0.1 (which may be too high), the amount accumulated during the ^{244}Pu mean life (1.2×10^8 yr) results in a concentration of 6.5×10^{-25} g g $^{-1}$ in the 10 km of the Earth's crust. The assumptions involved are the same as those in ref. 1. But the age of $\geq 5.5 \times 10^8$ yr for the bastnaesite used by Hoffman *et al.* needs to be revised to a more reasonable one of 1.4×10^9 yr in the light of a recent study by Fleischer and Naeser² and the biotite age determined by Lanpher⁸. The expected concentration of ^{244}Pu from cosmic rays is thus reduced to 5×10^{-30} g g $^{-1}$ in the crust which requires an enrichment factor for plutonium of about 2×10^{11} (20 million times that of cerium in the Earth's crust). A lower influx of cosmic-ray ^{244}Pu and a smaller survival factor for passage through the atmosphere would require a higher enrichment factor for plutonium in this bastnaesite.

The present estimate of cosmic dust influx is around 10^5 tons per year over the entire Earth's surface (S. Tanaka and colleagues, unpublished). The life history of the dust in space is not well known in spite of great concern to many investigators. But a rough estimate using the Poynting-Robertson effect, friction with interplanetary gases, light pressure and gravity as the forces exerted on the dust shows that a dust particle of less than $1 \mu\text{m}$ at 10^4 AU falls down to the Sun within a few thousand million years⁹. Assuming that some of the dust, say 10% of the influx on to the Earth, originated in frequent supernova explosions in our Galaxy and that the dust material is chondritic, with $[^{244}\text{Pu}/^{238}\text{U}] = 0.013$ and $[\text{U}] = 2.0 \times 10^{-2}$ p.p.m., the steady state influx rate of ^{244}Pu is expected to be 1.7×10^{-26} g ^{244}Pu cm $^{-2}$ s $^{-1}$. Using essentially the same assumptions as in the above cosmic-ray calculation, a concentration of 1.6×10^{-22} g ^{244}Pu g $^{-1}$ in the Earth's crust is deduced. This is almost the same as the average concentration in the crust obtained from the observation of Hoffman *et al.* using an enrichment factor for plutonium equal to that of cerium. The small difference of a factor of 0.6 may be not important, considering the present state of knowledge of ^{244}Pu behaviour from its place of birth to the ore formation, that is, the ^{244}Pu formation in supernovae, its distribution into interstellar medium, the condensation into dust grains, the dust collection efficiency by the Earth, the ^{244}Pu geochemistry and concentration mechanisms and the possible small differences in these properties between plutonium and cerium.

The reported ^{129}Xe excess of $(2.2 \pm 0.2) \times 10^{-12}$

cm 3 STP/g of natural iodyrite can be ascribed to the *in situ* decay of naturally occurring ^{129}I and leads to iodine with the isotopic composition of $3.3 \times 10^{-15} \geq ^{129}\text{I}/^{127}\text{I} \geq 2.2 \times 10^{-15}$ if extrapolated back to some 50 m.y. ago when the ore was formed³. The concentration of ^{129}I in the Earth's crust is thus expected to be $(1.2 \sim 13) \times 10^{-22}$ g g $^{-1}$ from the enrichment factor of $(5 \sim 36) \times 10^5$ based on the iodine content of 25% in the ore and the crustal iodine abundance of 0.07 to 0.5 p.p.m. (refs 10, 11).

If the cosmic dust carries 0.1 p.p.m. of iodine with an isotopic composition of $^{129}\text{I}/^{127}\text{I} = 1.1 \times 10^{-4}$ (chondritic values¹²), the global influx rate of 1×10^4 ton yr $^{-1}$ would correspond to an influx of 7×10^{-28} g ^{129}I cm $^{-2}$ s $^{-1}$ and the same processes mentioned above for ^{244}Pu in the dust would yield a concentration of 2×10^{-19} g ^{129}I g $^{-1}$ in the crust. This value is two to three orders of magnitude higher than the expected one from the ^{129}Xe observed in the iodyrite.

Iodine is known to concentrate in hydrosphere and biosphere rather than in lithosphere. Even in the crustal materials the iodine contents varies widely from type to type of rocks, for example $0.007 \sim 0.5$ (typically $0.046 \sim 0.1$) p.p.m. in igneous rocks which are the major crustal rock, $7 \sim 38$ (typically 10) p.p.m. in shales which are the major sedimentary rock and $10.9 \sim 49$ (typically 50) p.p.m. in deep sea sediments^{11,12}. The variation of iodine concentration between the Earth's surface and deep seated rocks thus ranges over factors of $10^2 \sim 10^4$ which compare reasonably well with the difference of ^{129}I between the expected values from cosmic dust and from the observed ^{129}Xe excess in the iodyrite.

Further contribution of ^{129}I to the Earth's constituents is expected from spontaneous and neutron-induced fission of uranium in rocks^{13,14}, spallation reactions on xenon in the upper atmosphere¹⁵, and neutron and muon reactions on tellurium^{16,17}. An estimate of the additions from these reactions has been obtained by Edwards and his coworkers to be about 2.2×10^{-12} for the marine $^{129}\text{I}/^{127}\text{I}$ ratio, which is within a factor of less than about ten of their experimental values for the Long Beach California brines, ranging from 5×10^{-12} to 2×10^{-11} , for assumed equilibrium ratios with hydrospheric and atmospheric processes at the time of trapping of ^{129}I (ref. 18). These results suggest an incomplete mixing of ^{129}I of the different origins with ^{127}I in various parts of the Earth.

An experimental search for ^{244}Pu in deep sea sediment would be feasible if the present hypothesis is valid. Further studies along this line are under way at our laboratory.

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Received October 16; revised November 30, 1973.

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New look at the lead isotope growth curve

THE isotopic composition of lead changes with time due to radiogenic production from isotopes of uranium and thorium. Two lead isotopes are produced from uranium, ^{206}Pb from ^{238}U , and ^{207}Pb from ^{235}U . The paired U-Pb decay schemes are particularly useful since geochronological information can be derived even when there has been a chemical fractionation of U from Pb at some time during the history of the sample. For example, the $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{207}\text{Pb}/^{204}\text{Pb}$ ratios can be measured in a galena (PbS) sample; by assuming that the parent material for the galena had suffered no chemical fractionation of U from Pb from the time of formation of the Earth until production of the galena, one can calculate a 'model' age of formation for the galena. This model is referred to as single stage growth of lead and can be characterised by a μ value [$(^{238}\text{U}/^{204}\text{Pb})$, as measured today] for the parent U-Pb system.

Stanton and Russell¹ suggested that conformable lead ore bodies may be expected to contain lead which would meet the conditions for single stage growth. They found that ores of different ages fitted approximately to a single growth curve and proposed that this curve represented the development of the lead isotopic composition of the lower crust or upper mantle with time. Using high precision analytical techniques Stacey *et al.*² redetermined the single stage growth curve and found that galena samples ranging in age from 0 to 3,100 m.y. fit a curve with $\mu = 9.09 \pm 0.06$.

The calculation of a single stage growth curve depends on a number of variables. First, the parent material for the galena must conform to the requirements of a single, chemically closed U-Pb system. Second, we must know the time at which the U/Pb ratio of the Earth became fixed (commonly referred to as the age of the Earth). Third, we must know the isotopic composition of lead at the time of formation of the Earth and, fourth, we must have accurate values for the decay constants of both uranium isotopes. We will assume for the moment that the first condition is met and look at the effect of the other variables on the calculation of model ages and model μ values.

The geological complexity of the Earth makes a direct measurement of its age of formation impossible. Using lead isotopes Tatsumoto *et al.*³ have measured the ages of several meteorites. The oldest age found was 4.57×10^9 yr (using the new uranium decay constants discussed below). This age is the same as the model lead age for the moon³, and provides the best estimate available for the age of formation of the Earth. Any change in the age of the Earth has the opposite effect to a change in the uranium decay constants since the two terms in the lead isotope growth equations are multiplied together [for example $(^{206}\text{Pb}/^{204}\text{Pb})_t = (^{206}\text{Pb}/^{204}\text{Pb})_{T_0} + \mu (\exp(\lambda T_0) - \exp(\lambda t))$].

The decay constants of uranium were recently determined using modern analytical techniques⁴. The new decay constant for ^{238}U is 0.92% larger than the previously accepted value⁵, whereas that for ^{235}U is 1.28% higher. For directly measured $^{206}\text{Pb}/^{207}\text{Pb}$ ages, such as the meteorite ages discussed above, the effect of the change in decay constants is rather small. At 4,500 m.y. the age difference resulting from recalculation of data with the new constants is -1.4%; at 3,000 m.y., -1.5%; at 1,500 m.y., -1.7% and at 500 m.y., -2.6%. Only at very young ages does the age difference become large. The situation for model ages, however, is quite different. Recalculation of a galena model age using the new decay constants results in a lowering of the model age by 200 m.y. at 3,000 m.y. and 350 m.y. at 600 m.y. model age. Although the largest shift in model μ values is for old samples, the biggest change in model μ values is for old samples. At 3,000 m.y. the newly calculated μ value will be 15% lower than the old value but at 600 m.y. the change is 8%.

Tatsumoto *et al.*³ reported new measurements of Canyon Diablo troilite lead isotopic compositions which are the most primitive yet found. Use of their troilite composition in the model age equation (rather than the values reported by Oversby⁶ results in a decrease in model age of about 100 m.y., regardless of the age of the sample, and lowers the model μ value by 2% for young samples, ranging up to a decrease of 6% for the oldest samples.

The combined effect of replacing all of the previously used physical constants in the model age equations by the more precise new parameters is a large shift in model ages and model μ values for all samples. The oldest samples have their model ages lowered by 300 m.y. (about 9%) and their model μ values decreased by about 20%; for the young samples, the model ages are lowered by 450 m.y. (50%-100%) and the model μ values are decreased by 10%.

Table 1 shows a selection of data from Stacey *et al.*²; the model ages and model μ values were calculated using the new parameters discussed above. Only the two oldest samples have model ages which are in reasonable agreement with the geological age of the surrounding rocks. These samples, which are our best candidates for single stage leads, have quite low model μ values. The youngest samples, whose model ages are about 500 m.y. younger than the age of the surrounding rocks, have considerably higher model μ values. This implies

TABLE 1 Model ages and model μ values for galenas

Locality	Geological age of surrounding rocks (m.y.)	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	Model age (m.y.)	Model μ value
Bathurst	460	18.20	15.65	Negative	n.a.
Balmat, New York	1,100	16.93	15.56		8.13
Broken Hill	1,500-1,700	16.01	15.40	1,210	8.12
Flin Flon	1,865	15.34	15.14	1,480	7.79
Geneva Lake, Ontario	2,500-2,700	14.00	14.87	2,340	7.89
Manitouwadge	2,700	13.21	14.40	2,630	7.39
Barberton	3,100	12.46	14.08	3,080	7.56

Isotopic composition data and ages of surrounding rocks from Stacey *et al.*². Constants used in model age calculation: $T_0 = 4570$ m.y.; uranium decay constants from Jaffey *et al.*⁴, $^{238}\text{U} = 0.155125 \times 10^{-9} \text{ yr}^{-1}$, $^{235}\text{U} = 0.98485 \times 10^{-9} \text{ yr}^{-1}$; initial lead isotopic composition³, $^{206}\text{Pb}/^{204}\text{Pb} = 9.307$, $^{207}\text{Pb}/^{204}\text{Pb} = 10.294$.

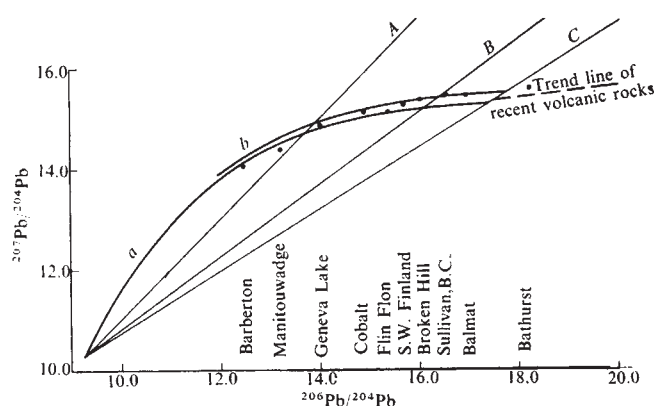


FIG. 1 Lead isotopic growth curves for $\mu = 7.8$ (a) and $\mu = 8.1$ (b), with isochrons for 2,500 m.y. (A) and 1,000 m.y. (B) and 0 m.y. (C). The galena data from Table 1 are plotted, together with data from Sullivan, British Columbia, South-west Finland, and Cobalt, Ontario. See Russell¹⁰ for references to the original data. The trend line for recent volcanic rocks¹⁰ is shown for comparison.

that the source region for conformable galena deposits has experienced an increase in its U/Pb ratio at some time between 2,600 m.y. and 1,600 m.y. The data are plotted together with a few additional analyses in Fig. 1. Single stage growth curves for μ values of 7.8 and 8.1 are shown for reference. The general trend of the data suggests a parent system which has had a small, rather steady increase in U/Pb ratio with time. This may reflect an increasing component of lead from upper crustal sources in the younger galena deposits, or may represent evolution of the lower crust and upper mantle.

Two apparent paradoxes associated with the single stage growth curve have disappeared by using the new decay constants. Several authors have argued that the age of the Earth must be considerably greater than 4,550 m.y. since old galena and feldspar samples contained lead whose model age was older than the geological age of the specimen^{7,8,9}. Using the most recent decay constants and initial lead composition for the Earth produces no cases in which model lead ages are older than the geological age of the samples, when either 4,550 m.y. or 4,570 m.y. is used for the age of the Earth.

The second apparent paradox concerned the comparison of lead isotope data from recent volcanic rocks with the galena model growth curve. Russell¹⁰ discussed the problem in detail and constructed a mixing model to try to resolve the paradox. Figure 1 shows the trend line for recent volcanic rock leads in relation to the galena growth curve. Using the old decay constants to construct the growth curve produced an intersection of the trend line with the geochron ($t = 0$ isochron), which fell at a much lower model μ value than that of the galena growth curve. Thus, one had to postulate a rather different history for the source regions of the volcanic rocks and the galena deposits. Use of the new decay constants produces an intersection of the volcanic trend line with the geochron at a model μ value of about 7.9. The volcanic data are better represented by a band of constant width with the same slope as the trend line. This band would intersect the geochron at model μ values of 7.8 and 8.0, very close to the range in model μ values indicated by the galenas. We may therefore postulate that the source regions of the volcanic rocks and the galenas have had similar histories in terms of their lead isotopic development, although the highest model μ values for the youngest galenas might suggest that the fractionation of U and Pb in the galena source regions either began earlier, or was somewhat more intense than the fractionation in the source regions for the volcanic rocks.

Considering the rather drastic changes brought about by the use of the new decay constants and initial lead isotopic composition, it is perhaps prudent to consider the error limits

on the present set of parameters used in model age calculations. The main chance of error in the initial lead isotopic composition would be if the troilite nodule analysed by Tatsumoto *et al.*³ contained a small amount of radiogenic lead as well as primordial lead. Lowering the initial $^{206}\text{Pb}/^{204}\text{Pb}$ from 9.307 to 9.214, and the $^{207}\text{Pb}/^{204}\text{Pb}$ from 10.294 to 10.236 would result in a change in model age of only 13 m.y. It is extremely unlikely that the initial lead composition was as low as these hypothetical values. It is possible that the age of the Earth might be slightly greater than the value of 4,570 m.y. used in the calculations above—although there is no positive evidence that the Earth is older than the Moon and meteorites. If we increase the age of the Earth to 4,600 m.y., the Barberton model age is lowered by 40 m.y. to become 3,040 m.y. and the Balmat model age is lowered by 125 m.y. to 460 m.y. The uncertainty in the decay constants of uranium is claimed to be less than 0.1% (ref. 4). This introduces an uncertainty of ± 7 m.y. in the model age of the oldest samples and ± 20 m.y. in the youngest model ages. There seems to be no likely source of error which would lead to a significant bias towards young ages among the model ages calculated with the new parameters. Pending availability of more data on meteorite ages, it is suggested that uncertainties in the model ages of old samples be assigned at between +10 and -40 m.y. and for young samples at between +20 and -125 m.y.

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Received November 20, 1973.

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Post-Cretaceous faulting in Sudan and its relationship to the East African Rift system

It has been widely believed that no relatively young faulting has taken place in Sudan since the Mesozoic. The national Geological Map of Sudan, scale 1:4 million, 1963 edition, and the International Tectonic Map of Africa, scale 1:5 million, 1968 edition, show no faults in the territory and indeed the most recent comprehensive reviews of the structure of Sudan^{1,2} indicate that the late Phanerozoic formations are not significantly affected by faults.

In northern Sudan the Nubian Sandstone Formation is the most extensive of the Phanerozoic strata. It is of Cretaceous age and comprises basal conglomerates, thick cross-bedded sandstones and mudstones. It has been stated¹ that no major faults affect this formation but that minor fractures with a displacement of only a few feet occur near Wadi Halfa and that minor faults affect sedimentary strata in the Red Sea Hills in the east of the country.

Recent geological mapping, geophysical investigations, and aerial photography and satellite imagery in various places across central and northern Sudan have indicated considerable faulting with significant downthrows. The faults can be recognised on the ground by stratigraphic dislocation, brecciation, iron mineralisation and silicification. The latter is highly developed along joint planes in the Nubian sandstones and both may be genetically related.

Evidence of faulting of the Nubian Sandstone Formation has been observed at several localities (Fig. 1), which can be summarised as follows.

(1) South of Khartoum. Along the White Nile valley older E-W and younger N-S to NNW faults with downthrows normally to E and NE occur, and at Jebel Aulia a NNW-trending fault has a displacement of more than 1,200 m^{3,4}. The partial effect of faulting and warping has been the Gezira depression, in which buried Nubian Sandstone Formation is preserved beneath Tertiary to Pleistocene sediments (Gezira Formation) up to 110 m thick.

(2) North of Khartoum. The sedimentary cover to the Sabaloka basement inlier is displaced by E-W and N-S faults^{5,6}, with probable rejuvenation along earlier planes of weakness in the basement.

(3) Central Sudan, Kordofan Province. At Bara village (Fig. 1) a NW-SE trough was revealed by gravity measurements and drilling^{7,8}. The depression is 50 km wide, at least 150 km long, and more than 400 m deep. It is filled with Nubian Sandstone Formation and Tertiary to younger fluvial sediments (Umm Ruwaba Formation; compare Gezira Formation).

(4) En Nahud to Fula, Kordofan Province. Geophysical work⁹ supported by drilling revealed faulted Nubian Sandstone Formation troughs trending NNW at Fula and probably forming a large NW-SE basin beneath Babanusa. Along the south edge of the sediment-filled Nahud outlier¹⁰ E-W faulting was indicated.

(5) Western Sudan, Darfur Province. Fault-controlled NW-SE depressions¹¹ near El Fasher (Fig. 1) contain more than 1,200 m of Nubian Sandstone Formation. Near Zalingei a broad zone of fractures displacing basement gneisses and lower Palaeozoic strata strikes NNW to NW and is associated with Tertiary to Recent volcanism¹². The zone is covered by recent sediments south of Babanusa.

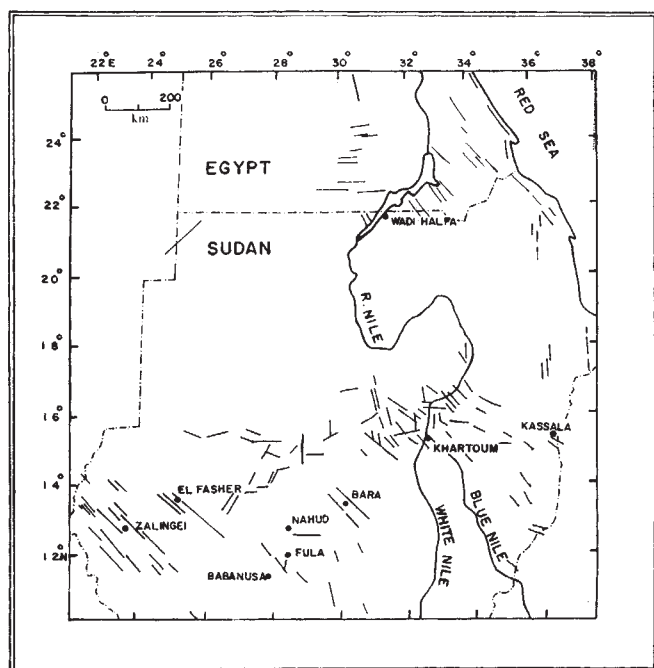


FIG. 1 Sketch map to show the distribution of post-Cretaceous faulting in Sudan and southern Egypt. Modified after Said¹⁸ and Vail²⁶.

(6) Eastern Sudan, Kassala Province. Faults displacing Nubian Sandstone Formation type sediments, the Gedaref Sandstone¹ and Tertiary basalts are recorded¹³⁻¹⁶ with general NW and NNE trends and with downthrows of up to 120 m.

(7) Northern Sudan and southern Egypt. Near Wadi Halfa faults trend E-W, NW or NNE¹⁷⁻²⁰. In Egypt faults trending N-S and NNW extend over 20 km with displacements up to 50 m (ref. 17). West of the Nile (Fig. 1) major faults cut Nubian Sandstone Formation and Upper Cretaceous strata; displacements are unknown.

(8) Red Sea coast. Tertiary sedimentary rocks along the coastal strip are affected by NNW, ENE and N-S faults^{18,21} with downthrow usually exceeding 50 m; total displacements may be considerable. Inland, faulting is difficult to recognise in the basement but satellite imagery indicates N-S to NW fracture patterns parallel to the Red Sea coast.

The age of the faulting may be indicated from the rocks affected. Across most of the northern half of Sudan the faults cut the Nubian Sandstone Formation of Cretaceous age, whereas in parts of eastern and western Sudan the faults displace Tertiary basalts. In southern Egypt E-W faults displace Nubian, Upper Cretaceous and lower Tertiary strata¹⁸. It may be assumed that most of the movement occurred during early Tertiary to more recent times. In Kordofan Province, for example, igneous activity of alkaline and carbonatitic character²² is apparently related to a fracture zone in which the most recent movement was only a few years ago²³.

The relationship between the post-Nubian faulting in the Sudan and the East African rift system is not known at present. The Egyptian geologists^{17,18,24} tend to consider a number of troughs or rifts to be present; however, there is some doubt whether these fractures can be directly related to rift valley tectonics²⁵ as has been claimed^{19,24}. Several of the fault depressions such as those at Jebel Aulia (just south of Khartoum), Bara and El Fasher are of the same order of magnitude as parts of the rift valleys of East Africa, and some have compatible trends. Several have been postulated as being structural features of the East African system^{7,18,19,24}. Unlike the East African rifts, however, the surface expression in Sudan is negligible, the grabens being for the most part filled by more recent sedimentary deposits, and buried beneath a superficial cover. The faulting has been assumed to have been mainly of Tertiary or Quaternary age and in this respect it is comparable to the main periods of rift faulting during the Miocene further to the south.

It seems therefore that the post-Nubian faulting in Sudan is broadly contemporaneous with the East African rift system, although morphologically not so evident at the surface, and that it probably resulted from related tectonic activity and associated volcanism that produced the East African system. Until more detailed mapping has been done, however, these fractures in Sudan should certainly not be referred to as rift valleys.

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BIOLOGICAL SCIENCES

5-Hydroxytryptamine accumulation in cerebrovascular injury

SEVERAL reports¹⁻³ indicate that some biogenic amines may significantly affect the intensity of the brain tissue reaction to injury, and that 5-hydroxytryptamine may be an essential factor in the production of brain oedema. This study was undertaken to explore this possibility, using as an experimental model brain oedema produced by the application of a cold metal plate to the exposed cerebral cortex of the cat⁴. This procedure results in severe damage to the upper cortical layers, including the disruption of blood vessels and the development of a vasogenic type of oedema. Vasogenic oedema is characterised by extracellular oedema fluid, containing extravasated serum constituents, spreading preferentially through the underlying and adjacent white matter⁵. It has been shown that the leakage of serum constituents is confined to the site of cortical injury, and that vascular permeability in the area of oedematous white matter itself, as tested for by protein tracers, remains unchanged⁶.

In the course of our investigations, we discovered that 5-hydroxytryptamine accumulates to a striking degree in the blood vessels in the region of cortical injury, although the oedematous white matter itself does not show any appreciable change in its 5-hydroxytryptamine levels. Here we describe this previously undescribed manifestation of nervous tissue reaction to severe injury.

Cold lesions were produced in cats by a procedure described previously⁴, and the animals were killed after 6 h. Corionally sectioned blocks of the brain, 2 mm in thickness and each containing the area of cold injury and adjacent gyri, were subjected to freeze-drying and processing according to the Falck technique⁷. Under the fluorescence microscope, the blood vessels within the area of cold injury showed a bright, yellowish-green fluorescence. Fluorescence of this nature was conspicuous in the walls of arteries, veins, and capillaries (Fig. 1). A few blood vessels showed an irregular, 'mottled' pattern of yellowish-green fluorescence in their lumina. Otherwise, the thrombotic material filling numerous blood vessels revealed only a faint bluish autofluorescence.



FIG. 1 Area of cortical cold injury, with blood vessels exhibiting a bright, yellowish-green fluorescence in their walls.

The characteristic yellowish-green fluorescence was not observed either in brain tissue adjacent to the fluorescent vessels or in blood vessels outside of the region of injury.

Microspectrofluorometry of the yellowish-green fluorescent vessels in the tissue sections revealed that this fluorescence had the excitation-emission spectra and photodecomposition rate characteristic of 5-hydroxytryptamine (Fig. 2).

The area of cortical cold injury and the underlying oedematous white matter were assayed for 5-hydroxytryptamine using a modification of the method of Maickel, *et al.*⁸ 5-Hydroxytryptamine in the injured cortical region was $329 \pm 30\%$ (mean $\pm$ s.e.m.) of that in the contralateral uninjured cortex; that in the underlying oedematous white matter remained at control levels.

Platelets seemed to be a potential source for the 5-hydroxytryptamine accumulating in the blood vessels. We tested this hypothesis by labelling platelet 5-hydroxytryptamine, producing cold lesions, and measuring the specific activity of 5-hydroxytryptamine in control and injured brain areas.

Whole cat blood (10 ml preserved with USP Formula A citrate dextrose) was incubated with ³H-5-hydroxytryptamine creatinine sulphate (specific activity 7.3 Ci mmole⁻¹), in a final concentration of 1.37×10^{-6} M, for 30 min at 37° C. Labelled blood was re-injected into the cat, and a lesion was made. Tissue 5-hydroxytryptamine was extracted into butanol and acidified heptane, after Maickel, *et al.*⁸ and counted by liquid scintillation. 5-Hydroxytryptamine in the injured cortex had a specific activity 1500% of that in the control cortex and of that in both oedematous and control white matter.

After intravenous injection of 100 μ Ci of ³H-5-hydroxytryptamine binolate (specific activity 1.0 Ci mmole⁻¹), 5-hydroxytryptamine in the injured cortical region had a specific activity 800% of that in control cortex, oedematous white matter, and control white matter.

The data suggest that a major portion of the 5-hydroxytryptamine accumulating in the area of the cold lesion is contributed by platelets. In addition, preliminary observations on cortical cold lesions in other species indicate a correlation between platelet 5-hydroxytryptamine content and the presence of fluorescent vessels. Species known to have appreciable quantities of 5-hydroxytryptamine in their platelets, such as rabbit, exhibit fluorescent vessels in the area of injury; species whose platelets contain little 5-hydroxytryptamine, such as the rat, show no fluorescence of vessels.

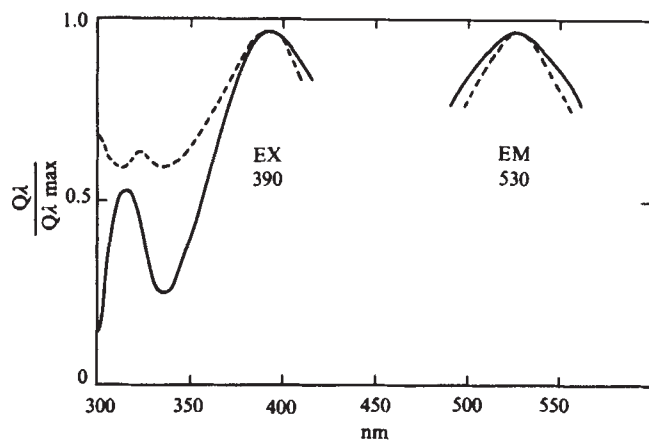


FIG. 2 Excitation (EX) and emission (EM) spectra of a fluorescent blood vessel (—), and of a 5-hydroxytryptamine oxalate sample (---) dissolved in buffered 1% gelatin at a concentration of 1 mg ml⁻¹. Microspectrofluorimetry was carried out using a modified Leitz fluorescence microspectrograph. A quartz optical system was utilised for obtaining excitation spectra¹².

The exact structural localisation of the 5-hydroxytryptamine observed in blood vessels remains uncertain because of the limited resolution of the light microscope. The lack of fluorescent material in the lumina of many fluorescent vessels suggests that platelets entrapped in luminal thrombi have released their endogenous 5-hydroxytryptamine. In this context, it has been shown that platelet 5-hydroxytryptamine is rapidly released during thrombus formation^{9,10}. It seemed, in some instances, as if 5-hydroxytryptamine was localised in the endothelial lining or muscular coat of the vessels. Presumably, it is sequestered cytoplasmically in such a manner that it is not degraded rapidly by mitochondrial monoamine oxidase.

The significance of 5-hydroxytryptamine accumulation in injured blood vessels remains to be ascertained. It is possible that this phenomenon may occur in vascular injuries other than those produced by cold, probably in those associated with thrombus formation. The possibility that 5-hydroxytryptamine acting on cerebral blood vessels may affect their permeability is supported by the recent observation of Westergaard and Brightman¹¹ that 5-hydroxytryptamine and other biogenic amines reaching arterioles and venules from the brain parenchyma may alter their permeability to protein tracers. It is conceivable that the presence of appreciable amounts of 5-hydroxytryptamine in injured blood vessels may significantly affect the duration and intensity of vascular leakage, which is a crucial factor in the dynamics of vasogenic brain oedema.

We thank Dennis L. Murphy of the National Institute of Mental Health for assistance in the labelling of the platelets.

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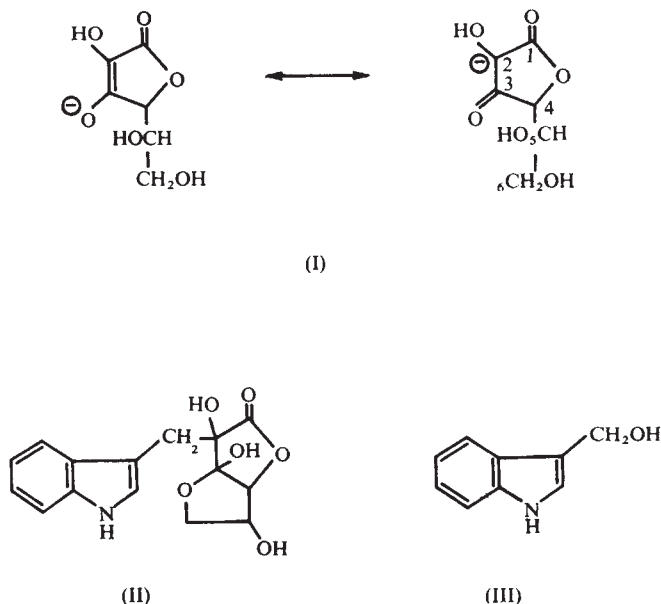
Ascorbic acid and biological alkylating agents

THE dangers to health from nitrite in the environment generally, and its use in meat processing in particular, have been pointed out by several groups¹⁻³. These dangers are associated with the production of nitrous acid in the acid environment of the stomach and the further reaction of this substance with naturally occurring and synthetic secondary and tertiary amines to form nitroso compounds which may be carcinogenic, mutagenic and cytotoxic. Ascorbic acid has been shown to effectively compete with amines for nitrous acid *in vitro*⁴ and it has been suggested that co-administration of ascorbic acid with potentially nitrosatable drugs, and foods containing nitrite, may reduce the hazards associated with ingestion of these substances⁴.

Kamm *et al.*⁵ have recently demonstrated the protective effect of ascorbic acid on the hepatotoxicity resulting from dual administration of sodium nitrite and aminopyrine to rats. Hepatotoxicity in this case is believed to be due to the formation of dimethylnitrosamine by reaction of nitrous acid with aminopyrine. They also reported⁵ that administration of sodium ascorbate, together with an oral dose of dimethylnitrosamine, partially prevented the liver damage produced by dimethylnitrosamine alone, thereby demonstrating an ascorbate-mediated protection which does not involve competition for nitrous acid. I wish to suggest a mechanism for the latter protective effect which may have general importance in the detoxification of environmental alkylating agents and in explaining certain aspects of their biological effects.

Many carcinogens, including dimethylnitrosamine, are believed to function as alkylating agents. They are either alkylating agents *per se* or they are metabolically converted into alkylating agents. A little-appreciated chemical property of ascorbic is its ability to react with alkylating agents⁶⁻⁹. At physiological pH, ascorbic acid (*pK_a*, 4.2) is largely in the anion form (I) and it is therefore particularly susceptible to alkylation *in vivo*¹⁰. The formation of ascorbigen (II) in plants¹¹⁻¹³ clearly illustrates the reactivity of ascorbic acid towards alkylating agents. Formation of ascorbigen involves alkylation of the ascorbic acid anion (I) by indole-3-methanol (III)⁸. This occurs very rapidly following the formation of indole-3-methanol in certain plant tissues in response to injury.

Under physiological conditions alkylation of the ascorbic acid anion (I) will occur mainly at C₂ of the ambident nucleophile to produce a new carbon-carbon bond, rather



than at the alternative oxygen site^{6,7} (compare ascorbigen). The carbon-carbon bond formed, unlike the carbon-oxygen bonds normally produced by the reaction of alkylating agents with other types of acid groups found *in vivo*, such as, carboxylic and phosphoric acids, will not be particularly susceptible to hydrolytic cleavage. Alkylation of ascorbic acid will therefore effectively compete with the alkylation of other cellular nucleophilic sites such as those on nucleic acids and proteins. It is this competition which I believe may provide a mechanism for the observed protective effect of sodium ascorbate on the hepatotoxicity of dimethylnitrosamine.

Ascorbic acid, if present in sufficient concentration, may also afford protection from the adverse effects of a number of other environmental alkylating agents by the same mechanism and the maintenance of high tissue levels of ascorbic acid may be of value for this reason. Some animal species, unlike man, are capable of synthesising ascorbic acid and this synthetic capacity, besides supplying the basic ascorbic acid requirement, may constitute a part of the metabolic defences of these animals. Thus a number of chemicals, including the carcinogenic hydrocarbons, 3,4-benzpyrene and 3-methylcholanthrene, are powerful stimulators of ascorbic acid synthesis in several animal species^{14,15}. The same chemicals are also potent stimulators of certain drug-metabolising enzymes in the liver¹⁵. Whereas the action of these enzymes detoxifies many harmful compounds, in some instances they convert chemicals to more toxic alkylating derivatives. The stimulation of ascorbic acid biosynthesis which accompanies the increase in activity of the drug-metabolising system may therefore represent an adaptation which, by providing a means of inactivating the alkylating metabolites produced by the drug-metabolising enzymes, would be of considerable benefit to the animal.

A extension of the above suggestion is that some of the biological effects of alkylating agents, which have in the past been mainly associated with their ability to alkylate nucleophilic sites on nucleic acids and proteins, may stem from the alkylation and rapid depletion of cellular ascorbic acid. It has been reported¹⁶ that a common feature of a number of carcinogens is their ability to degranulate the rough endoplasmic reticulum of cells¹⁶. Ascorbic acid deficiency in the fibroblasts of guinea pigs is characterised by a marked reduction in the extent of the rough endoplasmic reticulum, an increase in the number of free ribosomes and disorientation of the ribosomes which remain attached to the membrane^{17,18}. These abnormalities are reversed by

treatment with ascorbic acid, early changes being observed four hours after treatment with ascorbic acid and a return to an essentially normal state after 24 h^{17,18}. Thus ascorbic acid appears to be important for the normal attachment of polyribosomes to the endoplasmic reticulum and the degranulation of the rough endoplasmic reticulum associated with the action of carcinogens may, at least in some cases, be symptomatic of a rapid depletion of ascorbic acid.

Existing evidence supports the view that carcinogens will deplete animals of ascorbic acid. Smoking is reported to lead to a significant loss of ascorbic acid¹⁹. The carcinogen benzanthrone is known to markedly deplete guinea pigs of ascorbic acid²⁰ and some of the symptoms of benzanthrone toxicity have been successfully treated with ascorbic acid^{20,21}. Carbon tetrachloride, another carcinogen which is believed to act through a radical intermediate and which is known to degranulate the rough endoplasmic reticulum of hepatocytes¹⁶, provokes a marked depletion of ascorbic acid in the liver of rats²². Ross and Benditt¹⁷ have remarked on the similarity in appearance of the scorbutic fibroblast and the carbon tetrachloride damaged hepatocyte and ascorbic acid is reported to strongly protect guinea pig hepatocytes from carbon tetrachloride damage²³ suggesting that a rapid depletion of cellular ascorbic acid may play an important part in carbon tetrachloride toxicity.

There can be little doubt that a rapid depletion of cellular ascorbic acid will have an adverse effect on a number of cellular processes. The possibility that biological alkylating agents and some other chemicals such as carbon tetrachloride may be capable of this action should therefore be more widely known.

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Possible involvement of cyclic GMP in growth control of cultured mouse cells

NON-TRANSFORMED fibroblastic cells in tissue culture exist in one of two reversible growth states, a state of rapid proliferation (growing) and a state of relative quiescence (resting)^{1,2}. Transition from a resting to a growing state can be accomplished by a variety of mitogenic agents including growth substances in animal sera, insulin and proteases. 3', 5'-cyclic adenosine monophosphate (cyclic AMP) was implicated in this transition^{3,4} in that the intracellular concentrations of cyclic AMP in resting cell cultures fall after brief exposure to mitogenic agents^{3,4} and exogenous additions of high concentrations (10^{-4} to 10^{-3} M) of dibutyl cyclic AMP to the culture medium cause a partial reversal of the mitogenic response⁵. Kram and Tomkins⁶ have shown that exogenous additions of 3', 5'-cyclic guanosine monophosphate (cyclic GMP) to the medium of cultured mouse cells can counteract the inhibitory effects of dibutyl cyclic AMP upon some of the earliest events induced by a mitogenic signal (increased uptake of uridine, leucine, and 2-deoxyglucose). We now report (1) that relatively high concentrations (10^{-6} to 10^{-4} M) of cyclic GMP or its butyrate analogues when added to quiescent Balb/c 3T3 cultures can induce a substantial increase in DNA synthesis and (2) that within a few minutes of serum addition to quiescent cultures the intracellular concentration of cyclic GMP, as measured by two independent methods, rises by a factor of nine- to eleven-fold. Cyclic AMP and cyclic GMP concentrations were also measured throughout the remainder of the cell cycle.

Balb/c 3T3 cells obtained from Dr S. Aaronson were routinely grown in 10 ml of Dulbecco's modified Eagles medium (DEM) with 10% calf serum (Colorado Company) in 9 cm Petri dishes at 37°C under a 10% CO₂ (v/v) atmosphere⁷. For determinations of the intracellular concentrations of cyclic nucleotides monolayer cultures were quickly washed with Tris-buffered saline, 1 ml of 5% trichloroacetic acid (TCA) added per dish, and the contents immediately frozen on dry ice. Cultures were then stored frozen. Samples were thawed and centrifuged and the supernatant was extracted with ether and lyophilised to dryness⁸. The combined dry residues of ten Petridish cultures (1.5×10^7 to 2×10^7 cells) were dissolved in 1.5 ml of 0.1 M Tris-HCl (pH 8.5), 0.005 M MgCl₂ and half the samples were digested with 10 μ g ml⁻¹ 3', 5'-cyclic nucleotide phosphodiesterase to check the authenticity of the products assayed³. Both phosphodiesterase-treated and untreated samples were applied to 2 ml Dowex columns (Biorad AG 1-X8, 200-400 mesh, formate form) and the cyclic AMP- and cyclic GMP-containing fractions eluted by 2 N and 5 N formic acid respectively. Solutions were lyophilised to dryness and redissolved in 0.05 M

sodium acetate at either pH 4.0 or pH 6.2 for cyclic AMP- or cyclic GMP-containing fractions respectively. ³H-cyclic AMP and ³H-cyclic GMP were added after the initial thaw-

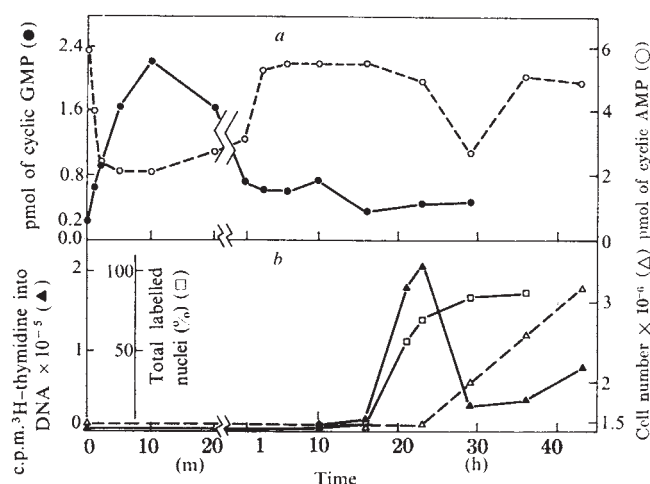


FIG. 1 Serum-induced sequential changes in cyclic nucleotide concentrations. *a*, Intracellular amounts of cyclic AMP⁹ (○) and cyclic GMP¹⁰ (●) were measured at different times after 20% serum and fresh medium addition to resting cultures of Balb/c 3T3 cells. Cell cultures were used 4 d after termination of DNA synthesis (less than 0.6% of the nuclei were labelled with ³H-thymidine in 18 h), and ten Petri dish cultures were isolated for each determination as described earlier. Similar results were observed if the Tris-buffered saline washing of the monolayer cultures was omitted. All results were corrected for differential losses during the isolation procedures (see earlier) and are expressed in pmol per 10⁶ cells. Duplicate results agreed to within 10%. Results after 3',5'-cyclic nucleotide phosphodiesterase digestion³ have been subtracted (0.1 and 0.3 pmol per 10⁶ cells for cyclic AMP and cyclic GMP respectively). Packed cell volumes and protein content per cell measured under these conditions do not vary more than 10% to 20% with changes in the cellular growth state¹², and thus the amounts measured reflect the relative intracellular concentrations. In the assays cyclic nucleotide amounts were measured in the range 0.1 to 1 pmol, and there was less than 1 part in 10,000 cross interference of cyclic AMP with the cyclic GMP radio-immune assay. *b*, Parallel cultures were also radioactively labelled with 3 μ Ci ml⁻¹ ³H-thymidine at 3 μ M for 2 h and the c.p.m. of ³H-thymidine incorporated into DNA (Table 1) recorded for a fifth of the cultures; the time-point represents the midpoint of the 2 h pulse-label (▲). Other cultures were also continuously labelled with 3 μ Ci ml⁻¹ ³H-thymidine at 1 μ M from 5 h after medium change until the time recorded for isolation (□) and then processed for autoradiography⁷. The fraction of cells with radioactively labelled nuclei was recorded (□). Cell numbers per 9 cm Petri dish were estimated in a Coulter Counter as previously described¹² (△).

TABLE 1 Changes in DNA synthesis with exogenously-added nucleotides

Concentration (μ M)	DB cyclic GMP	MB cyclic GMP	MB cyclic IMP	3', 5'-cyclic GMP	2', 3'-cyclic GMP	5'-GMP	DB cyclic AMP	cyclic AMP	cyclic CMP	MB cyclic UMP	cyclic UMP
2.5	10.2	21.6	5.7	5.8	-0.9	-0.7	0.6	1.2	0.7	1.2	1.1
25	20.3	22.5	28.4	5.0	0.2	-0.5	0.7	0.3	0.8	0.0	1.1
250	1.0	20.7	10.2	20.5	-1.0	-0.8	0.6	0.4	0.8	0.8	0.2

Results are expressed in thousands of c.p.m. of ³H-thymidine incorporated into DNA minus the value for control cultures with no additions (3,100 c.p.m.). Duplicate results agreed to within 15%. Abbreviations: DB, dibutyl; MB, monobutyl. Balb/c 3T3 cells were seeded in 5 cm Petri dishes at 10⁵ cells per dish in 5 ml of DEM medium containing 2% calf serum (v/v). Seven days later the medium was removed, cell monolayers washed twice with DEM alone, and fresh media containing 250 μ g ml⁻¹ bovine serum albumin, 0.5 μ g ml⁻¹ hydrocortisone (Calbiochem), and 0.1 ng ml⁻¹ of a purified pituitary growth factor¹¹ were added. After two further days (cell density 1.5×10^5 per dish) the various nucleotides (from Sigma and Boehringer) were added, and the cell cultures were radioactively labelled with 3 μ Ci ml⁻¹ methyl-³H-thymidine at 3 μ M from 12 to 30 h after nucleotide additions. Medium was then removed, cell monolayers washed twice with Tris-buffered saline and lysed with 2.0 ml of 0.5 N NaOH. One millilitre of the cell suspension was incubated 16 h at 37°C before the DNA was precipitated with 10% TCA (w/v) and 50 μ g carrier calf thymus DNA. Precipitates were collected on glass-fibre filters and analysed for radioactivity¹². Addition of 20% calf serum yielded 112,000 c.p.m. of ³H-thymidine incorporated into DNA. Autoradiography⁷ of parallel cultures showed that approximately 1%, 15%, 18%, and 85% of the cell nuclei became radioactively labelled with ³H-thymidine after no addition, or the addition of 25 μ M DB cyclic GMP, 25 μ M MB cyclic GMP, or 20% serum respectively.

TABLE 2 Relative intracellular amounts of ^{32}P -cyclic nucleotides

	Resting cells	Serum-activated cells	Ratio: (Activated cells)/(Resting cells)
^{32}P -cyclic AMP (c.p.m.)	2,960	1,160	0.39
^{32}P -cyclic GMP (c.p.m.)	6,850	63,000	9.2
Labelled Nuclei (%)	0.8	83	100
DNA Synthesis (c.p.m.)	980	97,300	99

Forty Petri dishes (9 cm) of resting Balb/c 3T3 cells in DEM (2×10^6 cells per dish) were radioactively labelled with $100 \mu\text{Ci ml}^{-1}$ ^{32}P - H_3PO_4 for 11 h. Fresh medium containing 20% serum and $100 \mu\text{Ci ml}^{-1}$ ^{32}P - H_3PO_4 were added to twenty dishes (serum activated cultures) and all cells were isolated 19 min later as described. ^3H -cyclic AMP and ^3H -cyclic GMP were added to standardise the recovery and to act as reference markers. Approximately 1.5×10^8 c.p.m. of ^{32}P -TCA-soluble material was recovered in each case. After lyophilisation following ether extraction the dry residues were dissolved in 0.5 N HCl and chromatographed over successive cationic³ and anionic (Fig. 1) Dowex columns to remove ^{32}P - H_3PO_4 and nucleotide di- and triphosphates. Cyclic AMP- and cyclic GMP-containing fractions (Fig. 1) were lyophilised and dissolved in 0.1 M ammonium acetate (pH 7.0). Samples were applied to Whatman 3 MM paper and the chromatogram developed in a solvent of 3.5 parts of butanol, 2.5 of acetone, 4 of 0.5 M ammonium acetate (pH 7.5) for 38 h. The chromatogram was cut into 0.5 cm strips and analysed for radioactivity; the ^{32}P -cyclic nucleotides were well separated from other nucleotides (distances from origin: cyclic AMP, 40 cm; cyclic GMP, 33.8 cm; deoxy-TMP, 27.2 cm; AMP, 18.4 cm), and comigrated exactly with the ^3H -markers. ^{32}P -c.p.m. in the cyclic nucleotide regions were recorded for duplicate experiments which agreed to within 15%. Total cyclic nucleotide ^{32}P -c.p.m. were corrected both for losses in handling (approximately 50% of the ^3H markers were recovered) and for ^{32}P radioactive decay. Counts per minute of ^{32}P -ATP (or GTP) isolated from either resting or 20 min serum-stimulated cells were approximately the same, and thus the intracellular specific activity of ATP (or GTP) was constant. Comparison of ^{32}P c.p.m. of cyclic AMP and cyclic GMP do not reflect their relative concentrations in the cell as the intracellular specific activities of their precursor molecules (ATP, GTP) are different for a 12 h labelling period¹². Parallel cell cultures were also radioactively labelled with $3 \mu\text{Ci ml}^{-1}$ ^3H -thymidine at 3 μM from 14 to 28 h after medium change and the cultures isolated for autoradiography⁷ or DNA synthesis analysis (Table 1). Results are expressed as the fraction of cells with radioactively labelled nuclei (%) or as the c.p.m. of ^3H -thymidine incorporated into DNA for a tenth of one Petri dish culture.

ing of the cultures to monitor recovery, usually 70% to 80% of the input was recovered with less than 10% cross contamination in the respective cyclic nucleotide fractions. Cyclic AMP was measured by the protein kinase binding assay of Gilman⁹, and cyclic GMP by the radioimmune assay of Steiner *et al.*¹⁰.

Balb/c 3T3 cells could be maintained in a resting state in the absence of exogenously added serum by addition of very low concentrations of a growth factor purified from bovine pituitary glands¹¹ (less than 1% of the cellular nuclei became radioactively labelled with ^3H -thymidine in 18 h). Various nucleotides and their butyrate analogues were then added and Table 1 shows the changes observed in the incorporation of ^3H -thymidine into DNA. Only 3', 5' cyclic GMP and its butyrate analogues together with monobutyl cyclic inosine monophosphate (cyclic IMP) yielded a substantial induction of DNA synthesis (eight to ten times the value in resting cultures); other nucleotides tested including cyclic AMP and 2', 3' cyclic GMP failed to do so. Results obtained exactly as described in Table 1 on addition of 250 μM monobutyl cyclic GMP; cyclic GMP; GMP; or no addition to resting cell cultures maintained for 16 h in DEM without growth factor and hydrocortisone were 16,040; 12,030; 2,680; or 2,150 c.p.m. respectively. Therefore, the purified growth factor and hydrocortisone were not essential for the cyclic GMP enhancement of DNA synthesis but were included only to maintain a constant number of viable cells over a period of several days¹¹. In their absence, cellular viability as measured by the number of attached cells rapidly decreased after 48 h, and hence the results in Table 1 with the hormones present were obtained with non-degenerating resting cell populations capable of complete growth activation after serum addition (Table 1). The kinetics of induction of DNA synthesis were identical for serum or monobutyl cyclic GMP addition to resting cultures: increases in DNA synthesis started at 14 to 16 h after additions (not shown). Addition of monobutyl cyclic GMP to quiescent cultures^{7,12} of Swiss 3T3-4A, Balb/c 3T3 mouse cells, or baby hamster kidney cells seeded in 1%, 2%, or 1% calf serum respectively also caused a two- to three-fold stimulation of ^3H -thymidine incorporation into DNA over control cultures.

As the addition of cyclic GMP caused a significant induction of DNA synthesis presumably a certain fraction of the cellular population was induced to leave the resting state and enter the growing state. We then measured the intracellular concentration of cyclic GMP using a radioimmune assay¹⁰ at different times after addition of fresh medium and

serum to resting cultures of Balb/c 3T3 cells. Figure 1 shows that in addition to the two- to three-fold fall^{3,4,8} in the concentration of cyclic AMP shortly after medium change the concentration of cyclic GMP rose by a factor of eight- to eleven-fold over the value in unstimulated cultures reaching a maximum after 10 min. Thereafter the cyclic GMP value gradually declined and remained constant (within a factor of 2), although the cyclic AMP concentration rose again after 1 to 3 h to reach its maximum value before DNA synthesis (Fig. 1). Similar results were obtained with the 3T3-4A mouse line of cells (not shown). We also confirmed the changes in the relative cyclic nucleotide concentrations by radioactively labelling cells with ^{32}P - H_3PO_4 and directly isolating the cyclic nucleotides by chromatographic procedures. Table 2 shows that 19 min after fresh medium and serum were added to resting cultures of Balb/c 3T3 cells the cyclic GMP concentration was increased nine-fold, while the cyclic AMP concentration dropped by a factor of $2\frac{1}{2}$ -fold.

Fast, transient increases in the intracellular concentrations of cyclic GMP have recently been reported in other culture systems after administration of a mitogenic or mitogenic-like signal to quiescent cell cultures (for example, phytohaemagglutinin and concanavalin A on human peripheral blood lymphocytes¹³; insulin on rat fat cells or liver slices¹⁴), whereas cyclic GMP itself has mitogenic action on rat thymic lymphoblasts¹⁵ and can reverse the dibutyl cyclic AMP inactivation of the immune response in precursors of antibody-forming spleen cells¹⁶. Here we have shown in the same culture system both the capability of relatively high (non-physiological) concentrations (10^{-6} to 10^{-4} M) of cyclic GMP to stimulate DNA synthesis in a significant fraction of quiescent mouse fibroblasts and large transient increases (ten-fold) in the concentration of intracellular cyclic GMP within a few minutes of exposure to a mitogenic signal (fresh medium and serum). Other events associated with transition from a resting to a growing state (increased protein and RNA synthesis¹²) are also stimulated by monobutyl cyclic GMP addition to quiescent cultures (unpublished results). Exogenous addition of cyclic GMP may exert its mitogenic action by direct interference with the putative cellular growth-controlling machinery or by other less direct mechanisms such as the activation of the intracellular cyclic AMP phosphodiesterase¹⁷ to reduce cyclic AMP concentrations in the cell. The findings reported here, however, pose the possibility that cyclic GMP may act as a positive signal¹³ while cyclic AMP acts as a negative signal^{3,4,8} in the cell for controlling the growth of cultured fibroblasts.

We thank Dr R. Dulbecco for encouragement and support, Miss Margaret Seeley for technical assistance, and Miss Susan Schultz for help with the autoradiography. This work was supported by fellowships from the Helen Hay Whitney Foundation to P. S. R., the Deutsche Forschungsgemeinschaft to W. E. S., and a grant from the National Cancer Institute to Dr R. Dulbecco. We thank Dr D. Gospodarowicz for a gift of purified pituitary growth factor.

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Received October 1, revised November 9, 1973.

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Possibility of inborn defect in isovalericacidaemia involving altered enzyme specificity rather than total inactivity

TANAKA, Isselbacher and colleagues¹ have described an inherited metabolic defect, isovalericacidaemia, which, though not fatal in their patients, leads, if untreated, to mental retardation and episodes of convulsion. As the name implies, the condition is characterised by high levels of isovaleric acid in the plasma. During periods of remission, this compound, which arises from the breakdown of dietary leucine, is excreted as a glycine conjugate. From the fact that only isovaleric acid accumulates and not butyric acid (from fat oxidation) or isobutyric or α -methyl butyric acids (from valine and isoleucine breakdown) Tanaka *et al.*¹ have concluded that there must be a specific isovaleryl CoA dehydrogenase that is non-functional in isovalericacidaemia. It had been assumed previously² that straight and branched-chain fatty acyl CoA compounds alike were oxidised by the green (that is, short-chain) acyl CoA dehydrogenase (see Fig. 1).

Sidbury, Smith and Harlan³ described an inherited neonatal condition in two unrelated families, the "odour-of-sweaty-feet" syndrome, in which the blood and urine apparently contained large amounts of hexanoate and butyrate. Symptoms of lethargy were followed by twitching, convulsions and death

within 2-3 weeks. Sidbury *et al.*³ attributed the condition to a faulty green acyl CoA dehydrogenase. Recent reanalysis of the origin samples by Ando *et al.*⁴ suggests that the original identification of the fatty acids was incorrect and that these also were cases of isovalericacidaemia. Ando *et al.*⁴ stress the need for rigorous identification of the characteristic compounds in establishing the existence of a new metabolic lesion.

Tanaka *et al.*^{5,6} noted several striking similarities between the symptoms of isovalericacidaemia and those of Jamaican vomiting sickness. The latter condition, frequently fatal, is not caused by an inborn error of metabolism but by the ingestion of a toxin, hypoglycin A, present in unripe 'ackee' fruits.^{7,8} Severe hypoglycaemia is accompanied in this condition by vomiting, and leads to coma. The hypoglycaemia has been attributed to abolition of a glucose-sparing effect in the under-nourished subjects through inhibition of fatty acid oxidation. Von Holt *et al.*⁹ suggested that methylenecyclopropane acetyl CoA, produced by the catabolism of hypoglycin A, might be an inhibitor of the short-chain acyl CoA dehydrogenase. Posner and Raben¹⁰ observed that hypoglycin also inhibits the oxidation of leucine, and Tanaka *et al.*⁵ have now shown that injection of hypoglycin A leads to greatly raised levels of isovaleric acid in the blood of rats. This they adduce in support of their contention that isovaleryl CoA dehydrogenase is a separate enzyme. They maintain that the coma in Jamaican vomiting sickness probably results from high levels of isovaleric acid rather than the low levels of glucose.

The view of isovalericacidaemia as an inherited deficiency of a specific and separate isovaleryl CoA dehydrogenase has already found its way into several respected textbooks. Bearing in mind, however, the strictures of Ando *et al.*⁴ with regard to chemical rigour, one should perhaps also proceed with caution in the biochemical interpretation of inborn errors of metabolism once the relevant compounds have been characterised. It is not impossible that a separate dehydrogenase exists for isovaleryl CoA, but so far no attempt has been made to purify it and demonstrate directly (1) that it is not the same as butyryl CoA dehydrogenase and (2) that one enzyme is absent or defective and the other present in isovalericacidaemia. The hypothesis also totally fails to explain the origin of the severe hypoglycaemia produced by hypoglycin A. It seems necessary, therefore, to re-examine critically the experimental evidence for the separate existence of isovaleryl CoA dehydrogenase.

In cases of isovalericacidaemia, during acute episodes the levels of isovaleric acid rise to about 30 mg per 100 ml, 500 times the normal figure. Levels of the other branched-chain fatty acids are not correspondingly elevated. In leukocytes from these patients the rate of oxidation of leucine to carbon dioxide is depressed ten-fold as compared with normal subjects. Rates of oxidation of other branched-chain amino acids were not measured.

Investigating the effect of hypoglycin A and its metabolites on rats, Tanaka *et al.*⁵ found that 0.7 mM α -ketomethylene-cyclopropyl propionic acid (KMCP), a transamination product, inhibited conversion of 2-¹⁴C-leucine to ¹⁴CO₂ in liver slices by 87% and led to accumulation of isovaleric acid and its glycine conjugate. Their combined concentration was increased 180-fold. In addition, however, 2.1 mM KMCP inhibited isoleucine oxidation by 20% and led to accumulation of α -methylbutyrate (increased twenty-fold). Hypoglycin A itself (1.4 mM) also inhibited oxidation of isoleucine, though less strongly than that of leucine. Only valine oxidation was unaffected by hypoglycin A at this concentration. Leucine (60 mg per 100 g) orally administered to rats, following injection of hypoglycin A (10 mg per 100 g), raised the plasma level of isovaleric acid nearly 200-fold over the uninhibited control. In the same leucine-loading experiment, however, the level of butyrate was also raised forty-fold by the inhibitor. Moreover, in a similar experiment with

for the existence of a separate enzyme.

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Received November 8, 1973.

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Dramatic early increase in uterine eosinophils after oestrogen administration

Two separate receptor systems for oestrogens, thought to be involved in independent mechanisms of oestrogen action, have been found to exist *in vitro* and *in vivo* in the rat uterus. These are the cytosol-nuclear receptor system and the eosinophil receptor system¹⁻³.

The cytosol-nuclear receptor system exists in the epithelial, stromal and muscular cells of the uterus². This receptor system is thought to be responsible, through a two-step mechanism⁴⁻⁶, for the genomic response, which involves an increased transcription of mRNA. The mRNA then codes for the synthesis of specific proteins and enzymes, a process which results in the true growth of the uterus. The genomic response is blocked by actinomycin D⁷⁻¹⁰, but is not counteracted by 11-oxygenated corticosteroids¹¹⁻¹⁴. Oestradiol-17 β has a much stronger affinity than oestriol for the cytosol-nuclear receptor system and accordingly oestradiol-17 β is the stronger oestrogen for the genomic response¹.

The eosinophil receptor system has been demonstrated *in vitro*^{1,15} and *in vivo*² in the uterine eosinophil leukocytes of the mature rat. The eosinophil receptors have a high affinity, a limited binding capacity and a great specificity for oestrogens, but not for compounds without oestrogenic activity^{1,16,17}. The uterine eosinophil receptor system is con-

sidered to be involved in some of the early oestrogenic responses in the uterus, such as water imbibition, increase in vascular permeability, histamine-releasing and oestrogen-priming effects^{1,18}. These effects are not blocked by actinomycin D¹⁹, but are diminished by conditions that produce uterine eosinopenia (such as 11-oxygenated corticosteroids and progesterone) and are enhanced under conditions that produce uterine eosinophilia (see ref. 18 for a review). Oestradiol-17 β and oestriol have similar affinities for the eosinophil receptors and accordingly, both hormones are strong oestrogens for the early oestrogenic responses¹.

The eosinophil leukocytes are not present in the uteri of immature or ovariectomised animals, and they are attracted to this organ under oestrogenic conditions^{16,20-24}. To understand the role and importance of the uterine eosinophils and the mechanism of eosinotaxis it is necessary to know the kinetics of the oestrogen-induced uterine eosinophilia at early times. Eosinophils have been reported to increase in the uterus 12 h after the parenteral administration of oestradiol benzoate to castrated mice or rats²⁵. The increase in uterine eosinophils at earlier times was, however, overlooked. Here we describe a dramatic increase in uterine eosinophils as early as 5 min after an intravenous injection of oestradiol to immature rats.

Female immature Wistar rats, weighing 50 g, were used in the present experiment. The solution of oestradiol-17 β in saline-ethanol was injected under ether anaesthesia in the jugular vein, using a dosage of 30 μ g per 100 g body weight. The control rats were similarly injected with equal amounts of saline-ethanol. The animals were killed at various times after the injection, and the uteri excised. The right uterine horn was used for biochemical studies and the left uterine horn was fixed in neutral formalin for subsequent histological studies.

The following parameters were measured for each animal: uterine wet weight, DNA²⁶, RNA²⁷ and protein²⁸ content, eosinophils per uterine section (counted on thirty to forty sections) and total number of uterine eosinophils. The latter was estimated by multiplying the average number of eosinophils per section by an appropriate factor, taking into account the thickness of the sections and the total length of the fixed uterine horn.

There is a dramatic increase in uterine eosinophils, clearly evident as early as 5 min after the intravenous injection of oestradiol to immature rats (Fig. 1). At this time, the total number of uterine eosinophils have changed from less than ninety in the controls to 1,150 in the treated animals. The count of eosinophils per section, as well as the total number of uterine eosinophils increases continuously until 12-24 h (Fig. 1), when the total number stabilises at about 150,000. After 24 h, the number of uterine eosinophils diminishes progressively.

The increase in the uterine wet weight first becomes evident 3 h after the oestrogen injection. Increases in the RNA and protein content first appear 6 h after oestradiol injection. None of the above responses are apparent 1 h after oestrogen injection (Fig. 1).

Examination of the histological material also shows that a large number of uterine eosinophils are found, at early times, attached to the wall of the uterine capillaries and small veins. The eosinophils first appear in the uterus in the mesometrial pole of the uterine horn, especially between the muscular layers and in the mesometrium itself. At later times, they are distributed homogeneously throughout the uterus, as previously described¹⁵.

The very early increase in uterine eosinophils after oestrogen administration is of striking importance in the elucidation of the role of eosinophils in the early oestrogenic response. The eosinophils began to appear in the uterus within minutes, that is, earlier than several other changes in the early parameters of oestrogen stimulation, such as the water

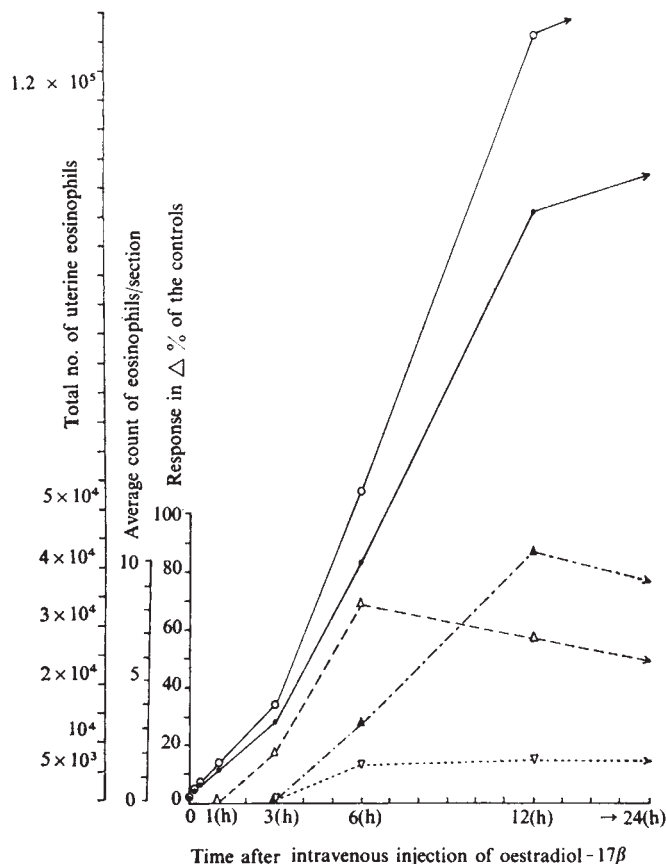


FIG. 1 Kinetics of oestrogen-induced uterine eosinophilia and other parameters of oestrogen stimulation. O, Total no. of uterine eosinophils; ●, average count of uterine eosinophils per section; Δ, uterine wet weight/DNA; ▲, RNA/DNA; ▽, proteins/DNA.

imbibition, histamine release and increase in RNA synthesis. An increase in cyclic AMP concentration is the only change reported at this very early time²⁹. Further data comparing the time sequences of early changes in cyclic AMP with the number of eosinophils in oestrogen-treated uteri are needed to discover if (and how) both parameters are related. The increase in cyclic AMP concentration has, however, been reported to be a transitory response²⁹, suggesting at least that the arrival of eosinophils in the uterus is independent of the continuous presence of cyclic AMP.

The rate of increase in the number of eosinophils in the present experiments suggests that the limiting factor of the oestrogen-induced uterine eosinophilia at early times, with the dose used in the present experiments, is the level of the eosinophils in the blood, which is low in the immature rat, and the production rate of eosinophils in the bone marrow.

The very early increase in uterine eosinophils after oestrogen administration suggests, although not conclusively, that eosinophils are directly attracted to the uterus by oestrogens. Previous *in vitro* and *in vivo* studies have demonstrated oestrogen binding by the uterine eosinophils^{1,2,15-17}. Autoradiographic studies of ³H-oestradiol *in vivo* have shown in eosinophils, besides the cytoplasmic localisation, the frequent appearance of silver grains along cytoplasmic interphases². This suggests the presence of a receptor for oestrogens near the cytoplasmic membrane of the eosinophils. *In vivo* studies have also demonstrated a higher concentration of labelled oestradiol along the walls of capillaries and small blood vessels in the rat uterus (unpublished data). The simultaneous presence of oestrogen receptors in the cytoplasmic membrane of the eosinophils and in the wall of the uterine capillaries and small blood vessels could be the key of the

organ-specificity of the oestrogen-induced uterine eosinophilia. It is possible that the oestrogens bound to the cytoplasmic membrane of the eosinophils also have affinity for receptors in the uterine capillary walls, thus allowing the attachment of the eosinophils to the capillary wall, which is the initial step towards penetration of the eosinophils into the uterus. The binding of oestrogens to the capillary wall may also induce conformational changes in its membrane which induces a specific attachment of the eosinophils to the capillaries. These hypotheses are under study.

This work was supported by a contract of the Ministry de la Politique Scientifique within the framework of the Association Euratom—Universities of Pisa and Bruxelles. A. T. is a Visiting Professor, and P. G. is a Maître de Recherches of the Fonds National de la Recherche Scientifique.

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2-Phenylethylamine as a possible mediator for Δ^9 -tetrahydrocannabinol-induced stimulation

If neuroamines modulate perception and affect, as implied in much of the literature, the behavioural effects of marihuana may be mediated by alterations of brain amine mechanisms. Even in large doses (5–500 mg kg⁻¹), Δ^9 -tetrahydrocannabinol (Δ^9 -THC) produces only relatively small changes in the brain levels and turnover of serotonin^{1–3}, catecholamines^{2–4} and acetylcholine⁵. We report here biochemical and behavioural experiments suggesting a role for endogenous 2-phenylethylamine (PEA) in the action of Δ^9 -THC. PEA may be one of the neuromodulators involved in wakefulness, arousal and excitement^{6,7}, and it is the precursor of phenylethanolamine, a putative neurotransmitter⁸. PEA is present in human⁸ and animal⁹ brain; monoamine oxidase B plays a major role in the metabolism of PEA whereas monoamine oxidase A is responsible for the deamination of noradrenaline and serotonin¹⁰. The observations that the urinary excretion of PEA is decreased in patients with endogenous depression^{11–13} and that in animals brain levels of PEA are increased by antidepressive treatments [monoamine oxidase inhibitors (MAOIs), imipramine, electroshock^{7,9,14}] suggest that this amine is important in modulating affect.

Brain levels of 2-phenylethylamine were determined in rabbits (New Zealand, male, 2–3 kg) using the method of Mosnaim and Inwang¹⁵, which measures the ultraviolet absorbance at 287 nm of the reaction product of PEA with ceric sulphate and HCl. For behavioural observation, ten male white Swiss mice (25 g) were grouped in a plastic cage [28 × 18 × 12 (height) cm]. Two observers used a simple checklist to evaluate behaviour in a 'blind' fashion. The number of experiments is listed in Tables 1 and 2.

Table 1 shows that 1 h after acute intravenous administration of Δ^9 -THC (3 mg kg⁻¹), brain levels of PEA had increased approximately four-fold. None of the other drugs studied in mice⁹, rats¹⁴, or rabbits¹⁶ induced such a large increase in PEA brain levels. Intravenous administration of 0.3 mg kg⁻¹ of Δ^9 -THC (once daily for 8 d) doubled PEA brain levels (note that the psychoactive dose of Δ^9 -THC in humans varies from 0.01 to 0.25 mg kg⁻¹ (ref. 17)). This increase in PEA brain levels may be due to a decrease in the rate of PEA disposition: in rabbits, Δ^9 -THC increased the amount of labelled PEA recovered from brain tissue following the intraventricular administration of trace amounts of labelled L-phenylalanine or of labelled PEA¹⁸.

Rabbits treated with Δ^9 -THC (0.3 or 3 mg kg⁻¹) showed a transient phase of excitement, followed by catalepsy with hyperexcitability to auditory stimuli (startle response was evoked by a soft clapping which evoked only an alerting response in untreated rabbits). Pargyline (100 mg kg⁻¹, given intraperitoneally, 72, 48 and 24 h earlier) increased the initial excitatory effect of Δ^9 -THC.

In mice, Δ^9 -THC (5 mg kg⁻¹) reduced spontaneous motor activity, and induced hyperexcitability and episodes of postural arrest with staring. In contrast, when mice were pretreated with the MAOIs nialamide (Table 2) or pargyline (100 mg kg⁻¹, 48 and 2 h earlier; or 100 mg kg⁻¹, 72, 48 and 24 h earlier), Δ^9 -THC increased motor activity and induced hyperexcitability and episodes of stereotyped behaviour ('popcorn effect') during which all the mice in the cage jumped, ran and vocalised. These episodes (lasting seconds or minutes) were separated by periods of relative quiet and occurred either spontaneously or were evoked by auditory stimuli. In the doses used, nialamide and pargyline inhibit brain MAO, and pargyline increases the levels of endogenous brain PEA¹⁶. Thus, the ability of these MAOIs to unmask this behavioural stimulant effect of

TABLE 1 Increase in brain levels of PEA induced by acute or chronic intravenous administration of Δ^9 -THC dissolved in 1 ml of Tween 80.

Treatment	mg kg ⁻¹	Time between injection and death (h)	Brain PEA mg kg ⁻¹ wet tissue $\bar{X} \pm s.e.$	No. of groups
None	—	—	0.42 $\pm$ 0.17	6
Tween 80	—	1	0.58 $\pm$ 0.20	6
Δ^9 -THC	3	1	2.30 $\pm$ 0.76*	6
Tween 80	Once daily, 8 d	24	0.66 $\pm$ 0.23	6
Δ^9 -THC	0.3 Once daily, 8 d	24	1.36 $\pm$ 0.36*	6

Each group consisted of six male white New Zealand rabbits (2–3 kg).

* $P = 0.01$ (treatment compared with control).

Δ^9 -THC suggests that the latter is mediated by endogenous monoamines. Of the monoamines that may mediate the stimulatory effects of Δ^9 -THC, serotonin is an unlikely candidate because its behavioural effects are mainly depressant^{19–21}; moreover, the serotonin depletor *p*-chlorophenylalanine (Table 2) enhances rather than prevents the stimulatory effect of Δ^9 -THC (after MAOI) (see also related observations in refs 22 and 23). Endogenous catecholamines seem to inhibit rather than to mediate the stimulating effect of Δ^9 -THC since the catecholamine depletor α -methyl-*p*-tyrosine unmask or enhances the stereotyped and aggressive behaviour induced by Δ^9 -THC in mice (Table 2). Our results suggest that PEA mediates Δ^9 -THC-induced stimulation. PEA readily crosses the blood-brain barrier and its amphetamine-like behavioural effects are potentiated by MAOIs^{7,9}. As in the case of Δ^9 -THC, the administration of

TABLE 2 Effect of Δ^9 -THC followed by PEA on grouped mice

Pretreatments	Treatments	Behaviour 'Popcorn effect'	Fighting	Activity
None	Solvent	0	0	—
	Δ^9 -THC	0	0	—
	PEA	0	0	—
	Δ^9 -THC + PEA	0	0	—
Nialamide (150 mg kg ⁻¹ , 48, 24 and 2 h earlier)	Solvent	0	0	+
	Δ^9 -THC	++	0	++
	PEA	+	0	+++
	Δ^9 -THC + PEA	+++	+++	+++
α -methyl- <i>p</i> -tyrosine (100 mg kg ⁻¹ 3 h earlier)	Solvent	0	0	—
	Δ^9 -THC	++	0	—
α -methyl- <i>p</i> -tyrosine (100 mg kg ⁻¹ 3 h earlier) + Nialamide	Solvent	0	0	0
	Δ^9 -THC	+++	+	++
DL- <i>p</i> -Chlorophenylalanine (150 mg kg ⁻¹ 72, 48, and 24 h earlier)	Solvent	0	0	—
	Δ^9 -THC	0	0	—
DL- <i>p</i> -Chlorophenylalanine (150 mg kg ⁻¹ 72, 48, and 24 h earlier) + nialamide	Solvent	0	0	+
	Δ^9 -THC	++	+	—

Δ^9 -THC was given in a dose of 5 mg kg⁻¹ and was followed 1 h later by 6 mg kg⁻¹ PEA. Mice were in groups of ten and each experiment was performed at least twice. All drugs were injected intraperitoneally (0.01 ml g⁻¹ of body weight) and dissolved in saline; Δ^9 -THC was dissolved in saline and Tween 80 (24:1 (v/v)). Controls were injected with drug vehicles; saline controls were not different from untreated animals and are omitted from the table. Behaviour was evaluated by two observers in a blind fashion using a simple rating scale. The 'popcorn effect' is described in the text.

PEA (10 to 50 mg kg⁻¹) alone (without MAOI pretreatment) induced depression preceded by a brief stimulation, whereas in mice pretreated with MAOIs (doses as described above), PEA induced amphetamine-like stimulation, 'popcorn' behaviour and, less frequently, aggressiveness. PEA (6 mg kg⁻¹) did not significantly alter the behaviour of mice not pretreated with MAOIs and, in those that were pretreated it induced hyperactivity and a weak and brief 'popcorn' effect. Moreover, the stimulant effects of Δ^9 -THC and PEA are synergistic. In mice pretreated with nialamide (Table 2), the combination of Δ^9 -THC (5 mg kg⁻¹) and PEA (6 mg kg⁻¹, 1 h after Δ^9 -THC) induced marked hyperactivity, 'popcorn' behaviour and vicious fighting (defensive and aggressive postures, chasing, and biting to the point of drawing blood). A similar interaction between Δ^9 -THC and PEA was obtained in mice treated with pargyline except that fighting was not observed.

These behavioural experiments suggest that the observed increase in the brain levels of PEA induced by Δ^9 -THC are associated with an increase in free PEA available to receptor sites and that this is responsible for some of the stimulant effects of Δ^9 -THC.

These results were presented in part at the second international symposium on drug addiction in New Orleans, March 1973. We thank Mr Sam Myles for technical assistance. Drugs were supplied by the National Institute of Mental Health (Δ^9 -THC), Abbott Laboratories (pargyline), Pfizer Laboratories (nialamide) and Merck Sharp and Dohme (α -methylidopa).

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Received June 27; revised December 6, 1973.

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Regeneration of the ventricular myocardium in amphibians

REGENERATIVE capacity of the myocardium in vertebrates is reported to be minimal¹⁻³. Mauro has advanced the thesis that the absence of satellite cells in the cardiac muscle is responsible⁴. Most recently, Oberpriller and Oberpriller have reported mitosis in adult newt myocardial cells 16 d after minor trauma⁵. Here we report observations on the adult salamander which indicate that cardiac regeneration of great competency occurs in this animal.

The adult aquatic stage of *Triturus viridescens* was used, animals were stored in pond water in glass aquaria at room temperature and were fed chopped liver and beef twice weekly. Anaesthesia was obtained with 1:1,000 Tricaine (Ayerst) and the heart was approached through a midline ventral incision, splitting the sternum and incising the pericardial sac. Between 30% and 50% of the ventricular myocardium was removed with iridectomy scissors traversing across the ventricular cavity (Fig. 1) and the skin incision was closed with interrupted 4/0 silk sutures. The animals were immediately transferred to fresh pond water in finger bowls and after recovery, returned to glass aquaria. No antibiotics or other chemical agents were used. During recovery, skin capillaries were observed microscopically in the tail fin, using transmitted light. For histological examination, the heart was removed *in toto*, fixed in buffered formalin with subsequent routine processing in hematoxylin and eosin. For electron microscopy, the hearts were fixed in cold 4% phosphate buffered glutaraldehyde, post-fixed in osmium tetroxide and embedded in Epon. Thin sections showing silver-grey interference colours were stained with uranyl

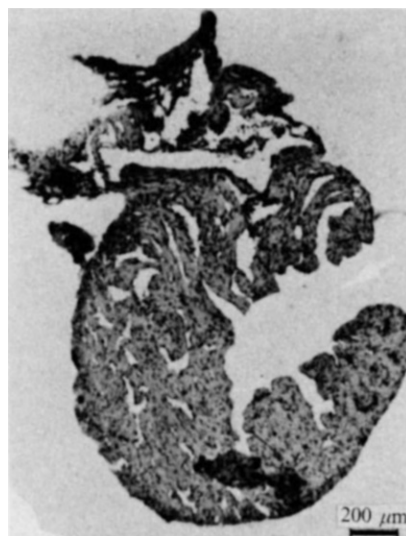


Fig. 1 Longitudinal sections of triturus heart showing extent of ventricular resection. Resection partially completed, entering into ventricular lumen from the right, in direction of the arrow. Excess portions of the auricles have been trimmed away after fixation.

acetate and lead citrate and examined with an RCA EMU-2D electron microscope.

In an initial series of twenty animals, all survived the procedure for two weeks after which they were killed. Since then, several hundred animals have been used for various phases of the study with an overall mortality rate of 10%. Observation of the heart *in situ* by dissecting microscope immediately after ventricular resection revealed pronounced contracture of the myocardium at the resection site resulting in a decrease in the diameter of the opening into the ventricular lumen. Bleeding was noted to be initially brisk, decreasing rapidly and ceasing with clot formation at approximately 60 s.

Complete circulatory stasis occurred within 5 min of resection. Erythrocytes were always trapped in the observed capillaries and after a variable period of time (30–120 min), oscillatory movement of these cells could be seen, with no directional flow. Directional movement (in which pulsations as such were not visible) began generally at 90 to 120 min but once occurred as early as 1 h. Circulation was noticeably slower than normal and generally did not become normal until 3–5 h after resection. Twelve animals were followed using smears (Giemsa-Jenner stained) of the peripheral blood at 30 min intervals following resection. At 2 h after resection, immature erythrocytes first began to appear and reached a maximum 15%–20% at 5 h.

Specimens for histological examination were taken at 5, 15, 30 and 60 min and 2, 3, 5 and 24 h after resection. Normal, non-operated specimens were processed at intervals to check on technique; no mitotic figures were observed in either the control or any of the experimental specimens. In the early experimental specimens, the major part of the clot was frequently lost in the process of removal and fixation of the heart. Many immature erythrocytes were visible, however, in the retained portions of the clot which were closest to the injured myocardium. After 30 min, the clot appeared to have differentiated into two zones, one closest to the injured myocardium which was composed of small basophilic cells with darkly staining nuclei and a peripheral zone of normal appearing erythrocytes with eosinophilic cytoplasm. After 3 h, the area of the resection was filled with a tightly packed mass of the basophilic cells (Fig. 2) and the original peripheral erythrocytes were undergoing

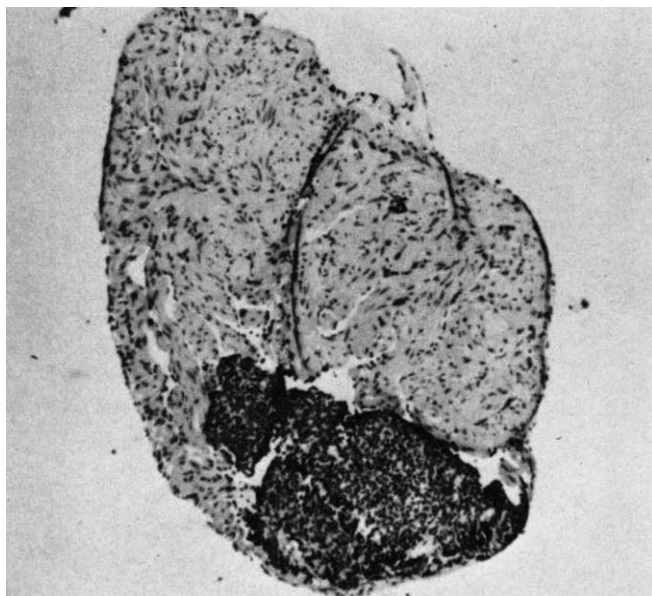


FIG. 2 Longitudinal section through ventricular myocardium 3 h after resection (magnification scale as Fig. 1). The mass of basophilic cells is clearly distinct, from the remainder of the myocardium and appears to be approximately the same in extent as the original resected portion.

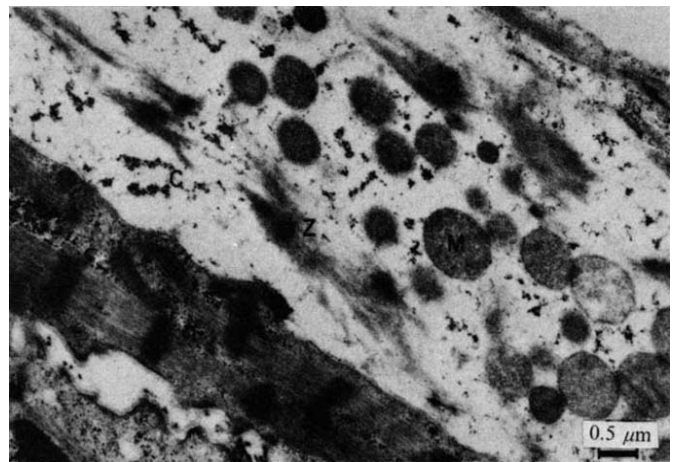


FIG. 3 Electron micrographs through site of ventricular resection two hours after resection; section through one of the cells bordering the defect, showing a region of active myofilament synthesis. Many polyribosomal complexes (C) are seen in close association with filamentous structures. z-band material and associated myofibrils (Z) are seen in the early stages of organisation. Mitochondria (M) show densely packed cristae and an electron-dense matrix and the sarcoplasmic matrix has a plaque embryonal appearance.

degeneration and nucleolysis. Between 3 and 5 h, the mass of basophilic cells diminished and slightly basophilic myocardial fibres appeared, which were arranged in a more open 'lattice-like' fashion than the remainder of the myocardium. By 24 h, sections appeared completely normal and there was no evidence of injury other than, occasionally, a small mass of degenerating erythrocytes exterior to the myocardium proper.

Specimens for electron microscopy were taken on the same time scale as for histological examination (except at 24 h). The early stages, 5 to 30 min, primarily showed the young forms of erythrocytes previously noted. The myocardium at the edge of the defect demonstrated several signs of degeneration in the 30 min specimen. By 2 h, the cells closest to the myocardial edge showed active fibrogenesis (Fig. 3).

Unquestionably, these animals can survive resection of up to 50% of the ventricular myocardium, with restoration of normal circulatory dynamics within 5 h. It seems that this is accomplished by some active cellular process with restoration of the missing myocardium rather than simple approximation of the cut edges and subsequent 'sealing off'. The origin of the reparative cells and the mechanism used to restore the myocardial mass is, as yet, unclear. If this represents a true regenerative process, it is more rapid and competent than any previously reported.

This work was supported by grants from the National Institutes of Health and the Veterans Administration Research Service.

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Received June 25; revised July 20, 1973.

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Thymine-labelled deoxyoligonucleotide involved in DNA chain growth in *Bacillus subtilis*

OKAZAKI *et al.* showed that bacteria labelled with short pulses of radioactive thymidine incorporate radioactivity into small DNA fragments, the so-called Okazaki pieces¹. These fragments have been observed in various bacterial, bacteriophage and eukaryotic systems^{2,3}. Pulse-chase experiments demonstrated that the Okazaki pieces are precursors of longer DNA chains, indistinguishable in length from the bulk of the DNA. Thus, the idea has arisen that DNA synthesis proceeds discontinuously on at least one of the two growing DNA chains of a given replication fork; that is, individual short DNA fragments are synthesised by a 5' → 3' DNA polymerase and then joined to the growing chain by ligase².

However, Werner has reported that cultures of *E. coli* labelled with short pulses of ³H-thymine incorporated radioactivity principally into large DNA; only a small fraction of the label was found in Okazaki pieces⁴. Furthermore, the fraction of the total label incorporated into Okazaki pieces increased rather than decreased with time of labelling during very short pulses. On the basis of these and other experiments, Werner concluded that DNA replication proceeds continuously without Okazaki pieces as intermediates⁴. Furthermore, he suggested that thymine and thymidine label separate precursor pools, thymine being used for replication and thymidine being incorporated primarily into Okazaki pieces involved in DNA repair⁵.

Using a thymine-requiring strain of *Bacillus subtilis*⁶, we decided to explore the possible differences between thymine (T) and thymidine (TdR) labelling of DNA. Cultures (80 ml) of *B. subtilis thy- trp-* were grown in minimal medium at 25°C until late exponential phase (1.5×10^8 cells ml⁻¹), centrifuged and resuspended in 5 ml of fresh medium containing 5 µg ml⁻¹ T. The concentrated cells were incubated at 25°C for 10 min and then labelled with short pulses of ³H-T or ³H-TdR. Pulses were terminated with -20°C acetone, the cells lysed and the lysates sedimented in alkaline sucrose gradients.

Figure 1 compares the labelling patterns of cells pulsed with ³H-T against ³H-TdR. In both cases, most labelled DNA sediments slowly with an S value of 30 or less. Very little radioactivity is found at a position in the gradient corresponding to 75S, the sedimentation coefficient of bulk DNA under our conditions. (The radioactivity in the final fraction of each gradient is due to unlysed cells.) Figure 2 shows the labelling patterns found with longer pulses of ³H-T. Again, most labelled DNA sediments more slowly than bulk DNA. The S value increases gradually as the pulse time increases, in a manner similar to that reported by Okazaki *et al.* for ³H-TdR labelling of *B. subtilis*². The gradual transition shown in Fig. 2 is characteristic of *B. subtilis*; a significant number of intermediate size chains are observed, suggesting that joining of Okazaki pieces may be slower in *B. subtilis* than in *E. coli*².

Results similar to those seen in Figs 1 and 2 are also obtained by using unconcentrated cells, or by using a differ-

ent solution to stop the pulse (10% pyridine-20 mM KCN - 1 mM EDTA at 0°C)⁷. In all cases thymine labels

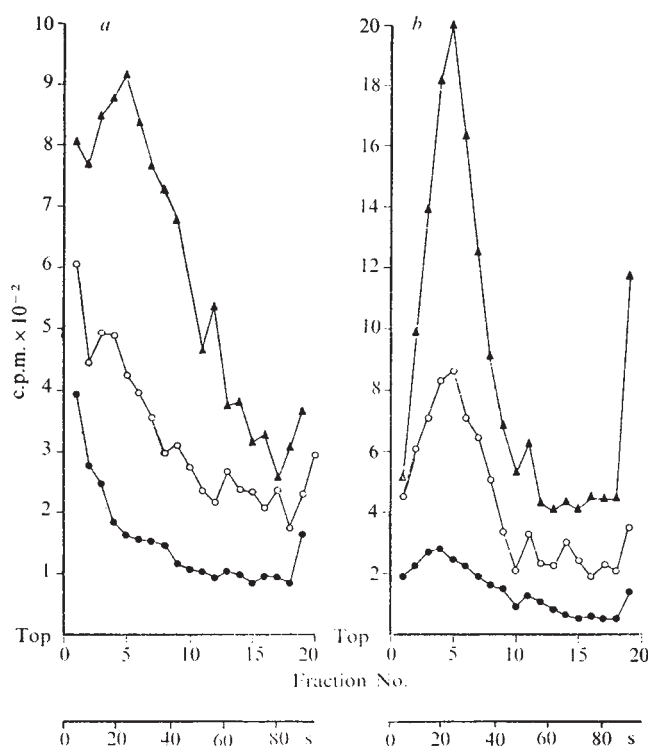


FIG. 1 Alkaline sucrose gradient sedimentation of pulse-labelled *B. subtilis* DNA. *B. subtilis thy- trp-* was grown at 25°C in a medium containing 0.2% (NH₄)₂SO₄, 1.4% K₂HPO₄, 0.6% KH₂PO₄, 0.02% MgSO₄·7H₂O, 10⁻⁶ M FeSO₄·7H₂O, 10⁻⁷ M MnCl₂·4H₂O, 0.2% glucose, 0.2% Na₂ citrate·2H₂O, 0.2% casamino acids, 40 µg ml⁻¹ tryptophan, and 5 µg ml⁻¹ thymine. The doubling time is 110 min in this medium. For a single pulse labelling experiment, 80 ml of cells was grown to late exponential phase (1.5×10^8 cells ml⁻¹), collected by centrifugation at 4°C, and resuspended in 5 ml of fresh complete medium. The concentrated cells were shaken gently for 10 min at 25°C, then stirred vigorously with a magnetic stirrer, and pulse labelled with either 0.6 ml ³H-T (20 Ci mmol⁻¹, 1 mCi ml⁻¹) or 0.6 ml ³H-TdR (6 Ci mmol⁻¹, 0.1 mCi ml⁻¹). The pulse was terminated by the rapid addition of 20 ml of -20°C acetone. The cells were centrifuged at 4°C and then resuspended in 20 ml of a solution containing SSC (0.15 M NaCl, 0.015 M Na₂ citrate), 20mM KCN, 1mM EDTA. The cells were centrifuged again at 4°C and the cell pellet suspended in 0.15 ml of a solution containing 27% sucrose, 20 mM KCN, 10 mM EDTA, pH 8.2. A 25 µl sample of 40 mg ml⁻¹ lysozyme was added and the sample incubated for 30 min at 37°C. Then 0.05 ml of 10% sarkosyl and 0.1 ml of 1 M NaOH - 0.1 M EDTA were added. After gentle shaking, the cells began to lyse; 0.7 ml of 0.3 M NaOH and 30 mM EDTA were added slowly. The entire lysate was layered on top of a linear 5% to 20% (w/v) alkaline sucrose gradient containing 0.3 M NaOH, 0.5 M NaCl, 10 mM EDTA. Sedimentation was carried out in a Beckman L2-65B ultracentrifuge with an SW27 rotor spun at 20,500 r.p.m. for 16 h at 4°C. The centrifuge tubes were fractionated from the top with an Auto Densi-Flow fractionator (Buchler Instruments) into 19 fractions of equal volume. Individual fractions were precipitated by addition of 0.3 ml 0.1 M Na pyrophosphate, 0.3 ml 3 mg ml⁻¹ T, and 4 ml cold 15% TCA containing 0.50 mM Na pyrophosphate. The samples were kept at 0°C for 20 min, and the precipitates collected on nitrocellulose filters (Matheson-Higgins Co., 25 mm diameter, 0.45 µm pore size) which had previously been soaked in 50 mM Na pyrophosphate, 200 µg ml⁻¹ T. The filters were rinsed with cold 3% TCA, dried, suspended in a toluene-scintillator solution, and counted in a liquid scintillation counter. The S scale was determined using a Φ29 DNA marker (S = 26). a, cells pulsed with ³H-T; b, cells pulsed with ³H-TdR. Pulse time: ●, 8s; ○, 15s; ▲, 30s.

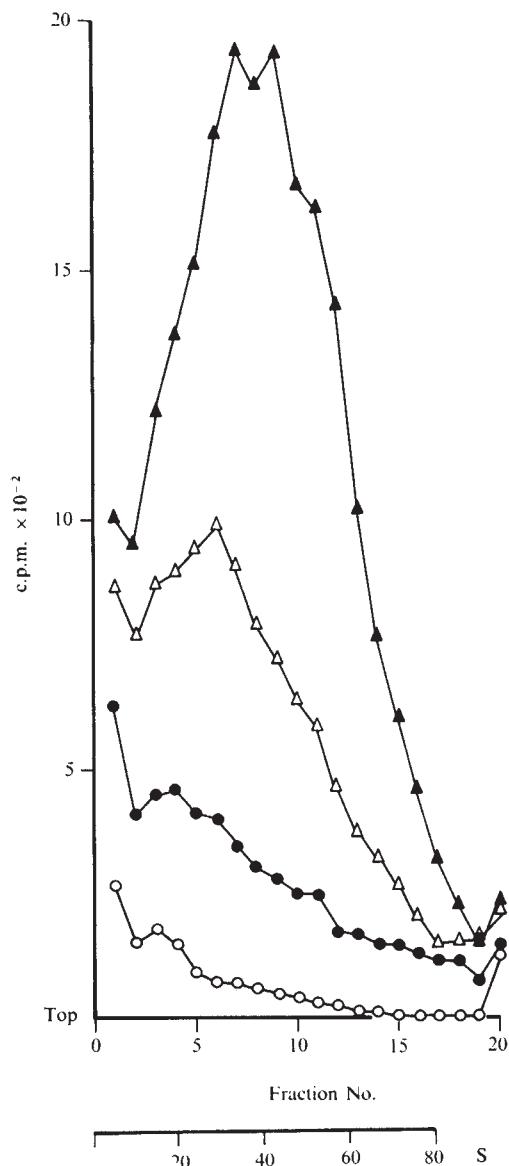


FIG. 2 Alkaline sucrose gradient sedimentation of pulse-labelled *B. subtilis* DNA ($^3\text{H-T}$). Conditions were as described in Fig. 1, except that $0.4 \text{ ml } ^3\text{H-T}$ ($12.6 \text{ Ci mmol}^{-1}$, 0.5 mCi ml^{-1}) was used for each sample. Pulse time: $\circ$, 15s; $\bullet$, 1 min; $\triangle$, 3 min; $\blacktriangle$, 6 min.

Okazaki pieces just as thymidine does, and the fraction of radioactivity in short DNA chains decreases rather than increases with the time of labelling. Pulse-chase experiments show that the thymine-labelled Okazaki pieces are precursors of larger DNA (data not shown). Independently, Okazaki *et al.* have shown that short pulses of $^3\text{H-T}$ are incorporated primarily into short DNA chains in both coliphage T4-infected cells and *E. coli*^{8,9}. Thus, there is no reason to doubt the common view that Okazaki pieces are intermediates in DNA replication, and that they can be labelled with either T or TdR.

We did observe one difference between T and TdR labelling of *B. subtilis*. In the case of $^3\text{H-T}$ labelling only, a significant amount of radioactivity can be seen in the top fraction (No. 1) of the sucrose gradients depicted in Figs 1 and 2. To separate the labelled material at the top of the gradient from Okazaki pieces, $^3\text{H-T}$ labelled lysates were sedimented for a longer time at a higher centrifuge speed. Figure 3 shows that a 15 s pulse of $^3\text{H-T}$ labels not only Okazaki pieces (fractions 9–14) but also a trichloroacetic acid (TCA)-precipitable component of about 2S (fractions 1–2). The 2S component is

labelled in very short pulses, before radioactivity appears in Okazaki pieces (Fig. 3).

We performed several control experiments to test if the rapidly labelled 2S component was actually a thymine-containing compound produced by live cells. First, we added $^3\text{H-T}$ to a non-radioactive lysate, centrifuged it through a sucrose gradient, and showed that there was no TCA-precipitable radioactivity in the gradient. Second, we noted that $^3\text{H-T}$, purified on a Sephadex G-25 column to separate it from potential macromolecular impurities, also could label the 2S component. Finally, we took fractions containing the 2S component from a sucrose gradient and treated them with formic acid, to hydrolyse the bases from the nucleic acids. Analysis of the hydrolysate by cellulose thin-layer chromatography demonstrated that the ^3H -labelled material co-chromatographed with a thymine marker, indicating that the 2S component is labelled with $^3\text{H-T}$ and not with a ^3H -labelled impurity.

To characterise the 2S component further, appropriate fractions were pooled from sucrose gradients, dialysed, and

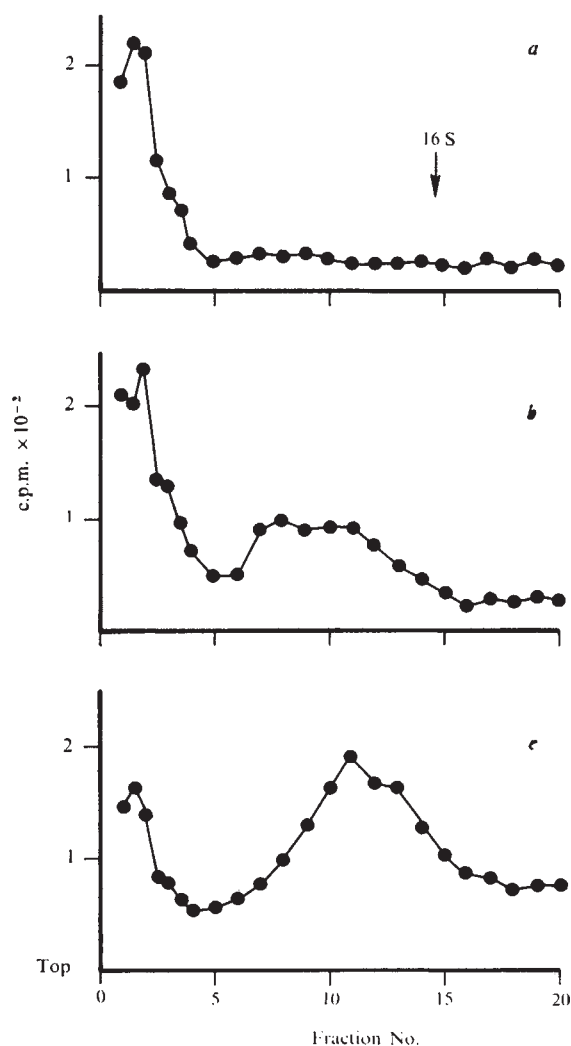


FIG. 3 Alkaline sucrose gradient sedimentation of pulse-labelled *B. subtilis* DNA. Conditions were as described in Fig. 1, except that $0.6 \text{ ml } ^3\text{H-T}$ ($12.5 \text{ Ci mmol}^{-1}$, 1 mCi ml^{-1}) was used for each sample, and sedimentation was carried out at 26,000 r.p.m. for 40 h. The 16S sedimentation marker, a gift of C. Mulder, was $^{32}\text{P-SV40}$ DNA converted to the linear form by the R_1 restriction enzyme¹⁵. The first six fractions collected from each gradient were half the usual volume, in order to improve the resolution. Therefore, the fraction indicated as No. 4 was actually the seventh collected. Fractions 4–20 were then collected in the usual manner. a, 3s; b, 8s; c, 15s.

subjected to treatment with various enzymes. In some cases Okazaki pieces were treated in a similar manner. Table 1 shows that the 2S component is degraded to TCA-soluble material by pancreatic DNase, snake venom phosphodiesterase, *E. coli* exonuclease I or hot TCA. However, it is resistant to RNase A, pronase or alkali digestion. This indicates that the 2S component is a deoxyoligonucleotide with a 3'-OH end. Furthermore, as Table 1 shows, the deoxyoligonucleotide is resistant to spleen phosphodiesterase, but becomes sensitive to spleen phosphodiesterase after digestion with bacterial alkaline phosphatase. Therefore, the 5' end of the deoxyoligonucleotide must be phosphorylated.

TABLE 1 Effect of various agents on 2S component

Agent	% degraded to TCA soluble	
	2S component	Okazaki pieces
Pancreatic DNase	83%	85%
RNase A	5%	—
0.3 N NaOH	5%	—
Pronase	15%	—
Hot TCA	90%	—
Exonuclease I	86%	—
Snake venom phosphodiesterase	80%	85%
Spleen phosphodiesterase	10%	6%
BAP	6%	9%
BAP + spleen phosphodiesterase	67%	50%

BAP, bacterial alkaline phosphatase.

The 2S component was obtained by pooling appropriate fractions from sucrose gradients similar to those shown in Fig. 3. Okazaki pieces were obtained from sucrose gradients similar to those shown in Fig. 1b. Samples were dialysed against either SSC (0.15 M NaCl, 15mM Na₃ citrate) or the solvents described below, and then treated as described below. Pancreatic DNase digestion, with 20 μ g ml⁻¹ enzyme, was performed at 37°C for 1 h in 10mM Tris, pH 7.6, 10mM MgCl₂. RNase A, heated at 80°C for 10 m at pH 5 in 0.15 M NaCl to inactivate contaminating DNases, was used at 50 μ g ml⁻¹, 37°C for 30 m. Pronase, self-digested for 1 h, was used at 1 mg ml⁻¹, 37°C, for 5 h. *E. coli* exonuclease I digestion was in a 1 ml reaction mixture containing 10mM MgCl₂, 1mM mercaptoethanol, 0.1 M glycine buffer, pH 9.4, and 35 U of enzyme, at 37°C for 1 h. Snake venom phosphodiesterase digestion was performed in 5mM MgCl₂, 10mM Tris, pH 8.0, with 160 μ g ml⁻¹ enzyme at 37°C for 1 h. Spleen phosphodiesterase digestion was in 0.3 M ammonium succinate, pH 6.5, with 2.5 U enzyme ml⁻¹ for 1 h at 37°C. BAP digestion was in 0.1 M Tris, pH 8.0, with 6 U BAP ml⁻¹ for 1 h at 37°C. When BAP and spleen phosphodiesterase were used together, the solvent was 0.3 M ammonium succinate, pH 6.5. The samples were incubated with BAP alone for 1 h, then spleen phosphodiesterase was added, and the incubation continued for an additional 1 h. Alkali treatment involved 0.3 M NaOH for 16 h at 37°C. Hot TCA treatment involved precipitating the sample in 5% cold TCA, and then heating it to 90°C for 30 min. In all cases, samples were precipitated with cold TCA, filtered, and assayed for radioactivity as described in the legend to Fig. 1.

In view of the importance of RNA priming for DNA synthesis^{10,11}, the deoxyoligonucleotide was tested for RNA attachment at its 5' end. It was subjected to strong alkali treatment (2 M NaOH for 16 h at 37°C), and then digested with spleen phosphodiesterase, with and without alkaline phosphatase. If the deoxyoligonucleotide had RNA on its 5' end, the alkali would have hydrolysed the RNA, leaving a deoxyoligonucleotide with a 5'-OH end. Then it would have become susceptible to spleen phosphodiesterase. Table 2, however, shows that the deoxyoligonucleotide remains resistant to spleen phosphodiesterase, even after alkali treatment, unless it is also treated with alkaline phosphatase. Therefore, the deoxyoligonucleotide does not have even a single ribonucleotide on its 5' end. If RNA was originally present on the 5' end, then the RNA must have been degraded by nucleases either before or during extraction of the deoxyoligonucleotide from the cell.

To estimate the size of the deoxyoligonucleotide, it was subjected to Sephadex gel filtration. Figure 4 shows that the deoxyoligonucleotide is eluted from a G-100 column at about

the same position as a tRNA marker. Based on these data,

TABLE 2 Effect of alkali on the 5' end of the deoxyoligonucleotide

Enzyme	% degraded to TCA-soluble	
	Control	Alkali predigestion*
Spleen phosphodiesterase	13%	23%
BAP + spleen phosphodiesterase	69%	63%

BAP, bacterial alkaline phosphatase.

*Alkali predigestion: 2 N NaOH at 37°C for 16 h. For other experimental details, see Table 1.

as well as on the fact that it is TCA-insoluble, we conclude that the chain length of the deoxyoligonucleotide is in the range of 50-100 nucleotides.

Figure 3 shows that the deoxyoligonucleotide is labelled to maximum radioactivity within 8 s. Since we know the specific activity of the ³H-T in the growth medium, and can assume steady state labelling, the number of deoxyoligonucleotides per cell can therefore be computed. We calculate that there are ten to twenty deoxyoligonucleotides per cell.

Is the deoxyoligonucleotide involved in DNA replication? The following evidence suggests that it is. First, two specific inhibitors of DNA replication, 6-(*p*-hydroxyphenylazo)-uracil (HPUra) and nalidixic acid, inhibit synthesis of the oligonucleotide. The experiment with HPUra is particularly significant because its mode of action is understood; the reduced form of the drug inhibits *B. subtilis* DNA polymerase III, an enzyme required for DNA replication^{12,13}.

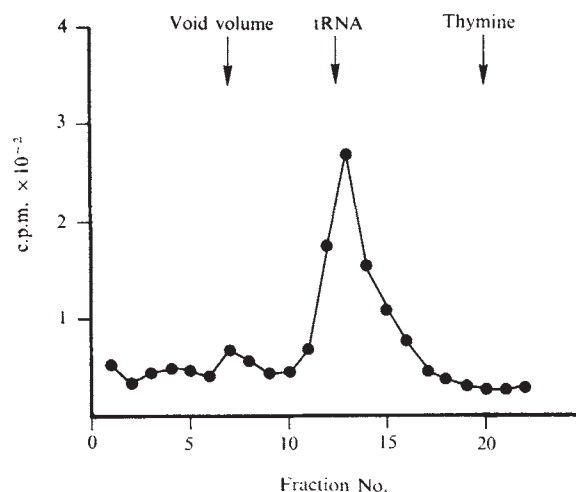


Fig. 4. Sephadex G-100 gel filtration of the deoxyoligonucleotide. Fractions containing the ³H-deoxyoligonucleotide were pooled from sucrose gradients similar to those shown in Fig. 3. They were neutralised and sedimented in CsCl to purify partially the deoxyoligonucleotide. Conditions of centrifugation in a Beckman L2-65B ultracentrifuge were as follows. The initial density of the CsCl was 1.7 g cm⁻³, SW65 rotor, 35,000 r.p.m. for 48 h at 10° C. The top 0.5 ml from each centrifuge tube was discarded and the remainder concentrated by dialysis against 30% polyethylene glycol. Finally, the deoxyoligonucleotide solution was dialysed against 0.1 M NaCl, 1mM Tris, 1mM EDTA, pH 7.0, and 0.2 ml applied to a Sephadex G-100 column equilibrated with the same solvent as above. The column size was 0.8 cm × 35 cm. Elution of the column with the solvent described above was at 25° C at 1 ml per 10 min. The void volume was determined using calf thymus DNA. The positions of wheat germ tRNA and thymine were determined by ultraviolet absorbance at 260 nm, and the position of the deoxyoligonucleotide by counting radioactivity as described in Fig. 1.

Pulse-chase experiments show that the deoxyoligonucleotide can be chased into larger DNA, although it is chased much more slowly (about 5 min) than it is labelled (Fig. 3). Because of the large deoxyribonucleotide pools in *B. subtilis* it is difficult to be sure that the oligonucleotide is chased directly into larger DNA. It could be broken down and the nucleotides re-used during the chase. There is an argument against breakdown and re-use; if cells are labelled with a short pulse of $^3\text{H-T}$ (which labels the deoxyoligonucleotide) followed by 5 min of incubation with 400 μM HPUra, the deoxyoligonucleotide is still found at its usual position near the top of a sucrose gradient. It is neither degraded nor chased into larger DNA. This suggests that the deoxyoligonucleotide is chased directly into larger DNA and not simply degraded and re-used during pulse chase experiments.

Hybridisation experiments show that the deoxyoligonucleotide hybridises with high efficiency, and equally well, to each of the separated strands of *B. subtilis* DNA; 11.3% of the radioactive deoxyoligonucleotide hybridises to the H (heavy) strand and 11.8% to the L (light) strand. (The separated strands were a gift of R. Rudner¹⁴.)

We do not understand why the deoxyoligonucleotide accumulates only after $^3\text{H-T}$ pulses (steady state labelling⁴) and not after $^3\text{H-TdR}$ pulses (non-steady state labelling⁴) in *B. subtilis*. Also, it is not observed after $^3\text{H-T}$ pulse-labelling of *E. coli* (our unpublished data and ref. 9). However, Jacobson and Lark, using T4 polynucleotide kinase to label the ends and hence count the number of chains in exponentially growing *E. coli*, found a large number of small DNA chains comparable in size to the deoxyoligonucleotide we observe⁷.

In summary, the following facts suggest that the deoxyoligonucleotide is involved in DNA replication. (1) It is labelled rapidly during pulses with $^3\text{H-T}$; (2) it can be chased into larger DNA, and (3) two inhibitors of DNA replication inhibit its synthesis. On the other hand, this evidence is also consistent with the possibility that the deoxyoligonucleotide is involved in DNA repair processes away from the replication fork. Further work is needed to determine the exact role of the deoxyoligonucleotide in DNA metabolism.

We thank Mrs J. Baird for advice, Dr N. Cozzarelli for *E. coli* exonuclease I, Dr C. Mulder for SV40 DNA, Dr R. Rudner for the *B. subtilis* DNA separated strands and the American Cancer Society for support.

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Received September 26; revised November 26, 1973.

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Protease inhibitors do not block transformed cells in the G₁ phase of the cell cycle

PROTEOLYTIC treatment releases normal fibroblasts from density-dependent inhibition of growth (DDI)^{1,2} and induces surface properties characteristic of transformed cells. Furthermore, increased³⁻⁵ and altered⁶ protease activities are found in transformed cells. Also, a number of commercially available protease inhibitors including N- α -tosyl-L-lysyl-chloromethane (TLCK) selectively inhibit the growth of transformed cells⁷. All this has led to the suggestion that a protease-like activity is required by transformed cells for unrestrained growth⁷ and, by inference, that inhibition of this activity should restore 'normal' growth control (that is, DDI). In fact, TLCK lowers the saturation density⁷, de-

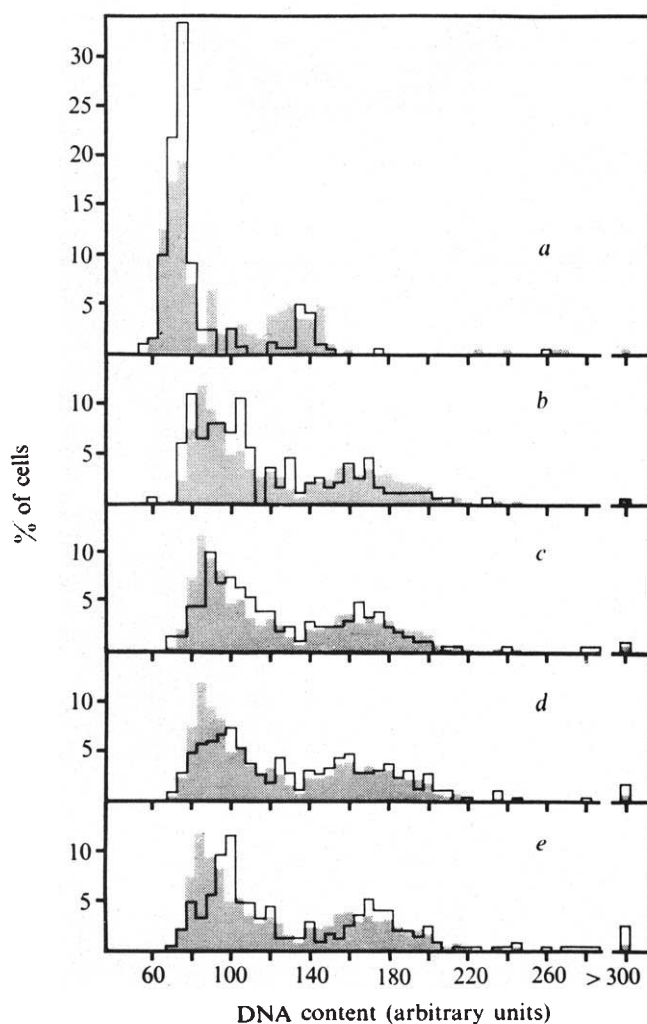


FIG. 1 DNA content of individual cells. a: 3T3 cells; shaded columns, log phase; open columns, at confluency. b-e: SV 3T3 cells; shaded columns, untreated controls; open columns, SV3T3 treated for 48 h with TLCK; b, 25 $\mu\text{g ml}^{-1}$; c, 50 $\mu\text{g ml}^{-1}$; d, 75 $\mu\text{g ml}^{-1}$; e, 100 $\mu\text{g ml}^{-1}$. All measurements calibrated with golden hamster lymphnode lymphocytes as reference (40 arbitrary units per cell).

TABLE 1 Effect of TLCK on proliferation and thymidine incorporation

Cell line	TLCK ($\mu\text{g m}^{-1}$)	Proliferation (doublings/24 h)		Mitosis per 10^3 cells	Thymidine incorporation	
		attached cells	total cells		c.p.m./ 10^3 cells (1 h pulse)	% labelled cells (4 h labelled)
SV 3T3	0	1.18 ± 0.20	1.19 ± 0.18	18.4 ± 3.8	125 ± 24	42 ± 4
	30	0.64 ± 0.23	0.66 ± 0.23	12.0 ± 2.5	119 ± 26	44 ± 5
	60	0.08 ± 0.07	0.17 ± 0.12	1.5 ± 1.4	56 ± 21	40 ± 2
	90	-0.21 ± 0.1	0.03 ± 0.03	0.1 ± 0.2	30 ± 20	42 ± 3
3T3 log phase	—	0.89	ND	12.6	105	32 ± 5
stationary phase	—	0.01	ND	0	1	2 ± 2

SV 3T3 cells were seeded in 3.5 cm Petri-dishes and allowed to grow to a density of approximately $10,000 \text{ cells cm}^{-2}$ (subconfluent). The medium was replaced and $20 \mu\text{l}$ of freshly made solutions of TLCK were added to each plate to give the final concentrations indicated. Thymidine incorporation was determined 24 h after the addition of TLCK, the mitotic and the labelling indices were determined 24 and 48 h after addition of TLCK. The number of cell doublings per 24 h was extrapolated from growth curves (5 points within 48 h). In addition to cells attached to the culture vessel, cells floating in the medium were also counted to give the total number of cells per plate. For comparison, untreated 3T3 cells in the log phase (about $20,000 \text{ cells cm}^{-2}$) and after reaching stationary phase were included in this experiment (five experiments with SV3T3; two experiments with 3T3). Figures in parentheses are % of the controls. ND, Not done.

creases the agglutinability with lectins and induces 'flat' morphology in transformed cells⁸. Here we present evidence that TLCK-inhibited cells are, however, not arrested in the G_1 phase of the cell cycle; thus, they do not meet a primary criterion⁹ for density-dependent inhibition of growth.

Freshly made solutions of TLCK (Calbiochem) were used for experiments. 3T3 mouse fibroblasts and their SV40 transformed derivative line (SV3T3) were used as the experimental cell lines. The procedures for the culture of the cells⁷, the growth inhibition studies⁷ and the thymidine incorporation⁸ have been published previously. Autoradiographs of cells grown on coverslips, labelled with ^3H -thymidine ($0.5 \mu\text{Ci ml}^{-1}$ medium; 5 Ci mmol^{-1}) for 4 h, were prepared with Kodak Bulk Emulsion NTB 2 and developed after 7 d exposure. To determine the mitotic index, cells were swollen and Orcein-stained according to the method of Fogh¹⁰. The DNA content of individual Feulgen stained cells was determined with an integrating microdensitometer (Barr and Stroud, Glasgow, Scotland). Two hundred to five hundred cells were measured per specimen at 560 nm , and the results expressed in arbitrary units. Lymph node lymphocytes from golden hamsters were placed on each of the microscope slides as reference cells.

The proliferation of SV3T3 cells is inhibited in a dose-dependent fashion by TLCK⁷; the inhibitory effect is mainly on cell proliferation and not simply due to detachment of cells from the plate (compare 'attached' and 'total' cells in Table 1), although there were some cells floating off into the medium, especially at high concentrations of TLCK. That TLCK inhibits proliferation is further supported by the fact that the reduced growth rate was paralleled by a decrease in the mitotic index (Table 1). The inhibition of cell proliferation was not, however, paralleled by a comparable decrease in DNA synthesis: even in TLCK-treated cultures where little or no net increase in cell number occurred, there was still considerable thymidine incorporation. Furthermore, the labelling index (4 h thymidine pulse) was not reduced by TLCK, although at higher concentrations of TLCK there appeared to be fewer grains (decreased by about 50%) over most of the labelled nuclei. By comparison, thymidine incorporation into 3T3 cells was drastically reduced after they became stationary (Table 1, bottom). Therefore, we conclude that TLCK does not stop transformed cells from entering the S phase.

This conclusion is further supported by measurements of the DNA content of individual cells in TLCK-treated cultures. In Fig. 1a, 3T3 cells are shown for comparison: there was a shift from the G_2 to the G_1 phase in 3T3 cells when they reached confluency. In contrast, in SV3T3 cells treated with increasing doses of TLCK (Fig. 1b-e), a shift to the G_1

phase did not occur. Rather, a dose-dependent shift to higher DNA content in the treated cells was seen. This seems to agree with the data in Table 1: the mitotic activity is reduced, but most cells continued to synthesise DNA (labelling index), although at a reduced rate.

In contrast to previous suggestions⁷, these results indicate that TLCK fails to induce density-dependent inhibition of growth in the sense that SV3T3 cells in the presence of this inhibitor are not arrested in the G_1 phase of the cell cycle.

We thank R. Bürk and Margaret M. Yarnell for helpful suggestions. We thank Dr H. Green of the Massachusetts Institute of Technology for the 3T3 and SV3T3 cells. A preliminary account of part of this work has been given at a recent meeting⁸. One of us (G. H.) is supported by the Swiss National Science Foundation.

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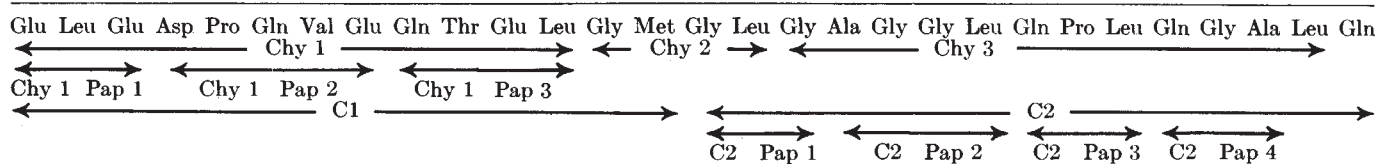
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The amino acid sequence of guinea pig C-peptide

THE discovery that the C-peptide of ox proinsulin is present in substantial amounts in the pancreas¹ has led the way to the study of C-peptides from a variety of mammalian species. The isolation and elucidation of primary structure has been described for the C-peptides of pig^{2,3}, man⁴⁻⁶, ox^{1,2,7,8}, dog, sheep and monkey⁹, rat¹⁰⁻¹², horse¹⁰ and duck¹³.

Fig. 1 Assignment of the primary structure of guinea pig C-peptide.



The horizontal arrows indicate the peptides liberated by the action of chymotrypsin (Chy) or cyanogen bromide (C) on the intact C-peptide and by the action of papain on the resulting fragments. The half arrows indicate amino acids released by stepwise degradation with carboxypeptidase A.

Fig. 2 Comparison of the amino acid sequence of the C-peptides of guinea pig and rat.

Residue number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Guinea pig	Glu	Leu	Glu	Asp	Pro	Gln	Val	Glu	Gln	Thr	Glu	Leu	Gly	Met	Gln	Leu	Gly	Ala	Gly	Gly	Leu	Gln	Pro	Leu			Gln	Gly	Ala	Leu	Gln
Rat I	Val					Pro		Leu					Gly		Pro	Glu		Asp			Thr		Ala	Leu		Glu	Val		Arg		
Rat II	Val					Ala		Leu					Gly		Pro	Gly		Asp			Thr		Ala	Leu		Glu	Val		Arg		

The primary structure of guinea pig C-peptide is that presented in this communication; the rat sequences have been reported^{10,12}. The horizontal line indicates residue common to the three structures. The differences between the three sequences appear to involve single base changes except at residues 8, 10 and 14 in the guinea pig. Residue 14 in the guinea pig is methionine whereas glycine occupies this position in the C-peptides so far reported.

The rodents, guinea pig, chinchilla and coypu, are classified in the suborder Hystricomorpha, principally on the basis of some anatomical and physiological characteristics. A knowledge of the primary structure of their proinsulins could contribute a molecular parameter to this classification. The amino acid sequence of their insulins is already known; the guinea pig sequence¹⁴ differs in 17 positions from that of coypu¹⁵ and coypu differs in 20 positions from chinchilla (N. R. Lazarus, R. Neville, and D. G. S., unpublished data). Elucidation of the primary structure of the corresponding C-peptides may provide further insight into the phylogeny of these species. Here, we present the amino acid sequence of guinea pig C-peptide.

C-Peptide was isolated from 2 kg (wet weight) of pancreatic tissue obtained from 350 Hartley strain guinea pigs. The method employed was essentially the same as that described previously⁴. The procedure yielded 8 mg of C-peptide and the homogeneity was assessed by analytical electrophoresis², NH₂-terminal analysis¹⁶ and amino acid analysis¹⁷. The sequence of amino acids was determined by quantitative techniques involving the isolation of fragments liberated by chymotrypsin, cyanogen bromide and papain (Fig. 1) and stepwise degradation of the individual peptides. Amides were assigned by comparison of the electrophoretic mobilities of peptides⁵ and by isolation of pyrrolidone peptides derived from NH₂-terminal glutamyl peptides.

The results allow a comparison to be made between guinea pig C-peptide and the only available rodent C-peptides, rat I and rat II (Fig. 2). The guinea pig sequence differs in 13 positions from rat I and 12 positions from rat II. Within the order Rodentia, the suborders Hystricomorpha and Rattus can be distinguished not only by their insulins but also by their C-peptides. It remains to be seen whether the structures of the hystricomorph C-peptides, when these are available, will support the existing hystricomorph classification.

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Received October 16; revised November 12, 1973.

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Second naturally occurring β -galactosidase in *E. coli*

SEVERAL studies have shown that the intestinal mucosa of various mammals contains at least two β -galactosidases¹⁻⁵. One of these has a primary specificity for lactose; the other has a primary specificity for synthetic β -galactosides such as *o*-Nitrophenyl β -D-galactopyranoside (ONPG). Since strains of *Escherichia coli* K12 bearing deletions of the *lac Z* gene have a very small residual activity toward ONPG, there must also be a second β -galactosidase in *E. coli*. We report here that *E. coli* K12 does indeed possess a second β -galactosidase. Although this enzyme has virtually no activity toward lactose, its synthesis is induced by lactose.

Strain DS4680A is a $lac^+ z^{del} y^+$ strain. It is accordingly unable to ferment lactose and will not grow on lactose as a sole carbon source. Table 1 shows the amount of β -galactosidase activity in crude extracts of DS4680A grown in proline + IPTG (a gratuitous inducer of the *lac* operon) and grown in proline + IPTG + lactose. (IPTG is isopropyl 1-thio- β -D-galactopyranoside.) The β -galactosidase activity is induced to increase 230-fold by lactose. Since DS4680A had undergone no selection for β -galactosidase activity, this observation suggested that the activity occurs generally in *E. coli* K12 strains. We accordingly tested two additional *lac Z* deletion strains for the presence and inducibility of this activity. The tested strains (see Table 1) are unrelated to either DS4680A or to each other. In each there is a β -galactosidase activity induced by lactose (but not by IPTG), supporting the hypothesis that the gene for a second β -galactosidase does occur normally in *E. coli*.

TABLE 1 Specific activity of β -galactosidase in crude extracts of *lac Z* deletion strains

Strain	Uninduced	Induced	No. of experiments
DS4680A	0.131	30.2	3
W4680	0.112	20.9	1
2320M15	0.023	13.0	1
1011	<0.0015	<0.0015	1

Cultures were grown in phosphate minimal medium containing 0.1% proline as a carbon source, vitamin B₁, 2×10^{-4} M IPTG (uninduced) or with the addition of 0.2% lactose (induced). The cells were collected and ground with alumina and the nucleic acids precipitated with streptomycin sulphate. β -galactosidase activity was assayed at 37°C in 5 mM ONPG in 0.125 M KPO₄ buffer, pH 7.5, containing 5 mM Mg²⁺. One unit of activity is defined as the hydrolysis of 1 nmol of ONPG per min. Protein concentrations were determined by the method of Lowry *et al.*⁸ Tabulated values are in U mg⁻¹ protein. All strains are *E. coli* K12. All bear deletions within the *lac Z* gene and have functional *lac* permeases (*lac Y*⁺). For purposes of comparison with the above values we have measured the specific activities of extracts of the wild type progenitor of DS4680A under these same conditions. The specific activity of an uninduced culture was 8.02 U mg⁻¹ protein; the specific activity of a culture in which the *Z* gene product was induced with IPTG was 11,865 U mg⁻¹ protein.

β -galactosidase II must have virtually no lactase activity *in vivo* since cultures in which the enzyme has been fully induced are not capable of growth when resuspended in lactose + IPTG minimal medium. The cells do have ample activity as measured by the hydrolysis of ONPG (10–30 U mg⁻¹) because strains can be selected that grow on lactose with as little as 16 U mg⁻¹. The enzyme is not cryptic *in vivo* because whole cells in which the β -galactosidase II activity has been induced are capable of hydrolysing ONPG. Therefore the failure of the cells to grow is probably due to an insufficient lactase activity.

β -galactosidase II has a small detectable amount of lactase activity *in vitro*. An extract of DS4680A in which the β -galactosidase II activity had been induced was prepared as in the legend of Table 1. The extract was permitted to hydrolyse lactose at a concentration of 139 mM under conditions identical to those described in Table 1. The reaction was terminated by heating for 10 min at 90°C. The amount of free galactose was assayed by the Galactostat assay system (Worthington), which uses galactose oxidase coupled with a chromogen. A unit of lactase activity is defined as the release of 1 nmol of galactose per min at 37°C. The specific activity of the extract toward ONPG was 41 U mg⁻¹ protein and toward lactose was 0.37 U mg⁻¹ protein. This small amount of lactase activity detected *in vitro* is clearly not sufficient to permit the growth of cells in lactose.

By a procedure similar to that of Campbell *et al.*⁶ we have obtained derivatives of strain DS4680A that contain an evolved β -galactosidase (*ebg*⁺) which permits growth on

lactose as a sole carbon source⁷. A *lac*⁻ revertant of one of these strains was found to have lost both the ability to grow on lactose and the second β -galactosidase activity (see strain 1011 in Table 1), although it retains a functional *lac* permease. The simultaneous loss of both lactose fermentation and β -galactosidase II activity could possibly be the result of a double mutation. We have shown that *ebg*⁺ is closely linked to the marker *tolC* at 59 minutes on the *E. coli* map, there being 18.7% recombination between *tolC* and *ebg*. We crossed strain 1011 to the F⁻ *lac Z*^{del} Y⁺ str^r *tolC* strain DLH-11 and selected str^r *tolC*⁺ recombinants. Sixty-eight such recombinants were scored for the presence of the inducible β -galactosidase II. Thirteen of the sixty-eight had such activity, giving a recombination frequency of 19.1% between *tolC* and the gene for the second β -galactosidase. Therefore the genetic position of the gene for the second β -galactosidase is the same as that of *ebg*⁺.

The function of the second β -galactosidase in both *E. coli* and in mammals is unknown¹, though there is the interesting possibility that their functions may be analogous.

This work was supported by grants from the US National Science Foundation, the National Institutes of Health and the University of Minnesota graduate school. B. H. is supported by a fellowship from the National Institute of General Medical Sciences.

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Received October 10; revised November 21, 1973.

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Detection by DNA polymerase I of breaks produced in rat liver chromatin *in vivo* by alkylating agents

In previous papers it has been shown that the *in vitro* template activity of DNA¹ and chromatin² with DNA polymerase I of *Escherichia coli* is altered by the action of ionising radiation. These changes have been attributed¹ to the formation of new binding sites on the DNA for the enzyme, some sites being active in the synthesis of DNA and others inactive. These new sites are associated with single-strand breaks in the DNA. By working under conditions either of enzyme excess or of excess of template it is possible to distinguish between newly formed active and inactive enzyme binding sites.

These studies^{1,2}, along with the work of Kornberg *et al.*³ have shown that DNA polymerase I could be of value in the investigation of single-strand breaks in DNA under non-denaturing conditions (as opposed to the denaturing conditions used in the alkaline sucrose gradient centrifugation technique). Many agents lead ultimately to single-strand breaks in DNA. Here we wish to report the use of DNA polymerase I to investigate the *in vivo* formation of single-strand breaks in the DNA of rat liver chromatin following injection of two different types of methylating agent, namely dimethylnitro-

samine (DMN) and methyl methanesulphonate (MMS). DMN is a known hepatocarcinogen in rats, producing tumours when fed as part of the diet⁴, or when injected as a single dose to neonates⁵ or to partially hepatectomised animals⁶; MMS is not known to produce liver tumours⁷. A notable feature of the present study is that after injection of these agents single-strand breaks can be detected even after very short times and after very low doses.

Chromatin was prepared from rat liver at various times following injection of known amounts of the methylating agent. Chromatin prepared from untreated rats was used as a control. The rate of incorporation of ³H-dTTP into acid-insoluble material was used as a measure of the rate of DNA synthesis and this was taken as a measure of the template activity of the chromatin.

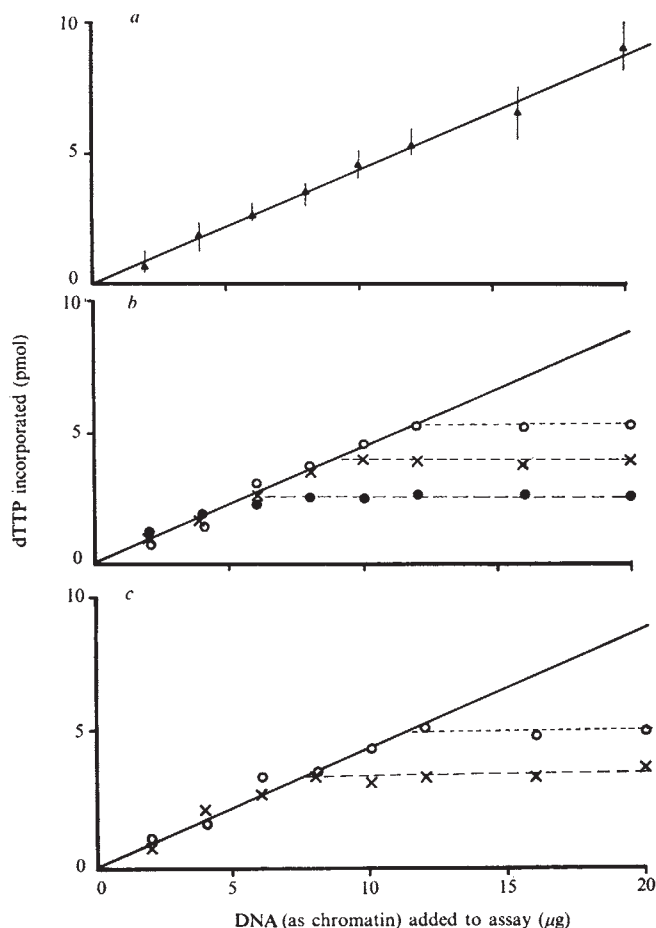


FIG. 1 Incorporation of dTTP into acid-insoluble material with increasing amounts of DNA (as chromatin) added to the assay. *a*, Control chromatin $\blacktriangle$; average of several independent experiments. The vertical lines indicate the range of values obtained. *b*, Chromatin following dimethylnitrosamine (DMN) treatment. The solid line shows control chromatin from *a*; $\circ$, 2 mg kg⁻¹; $\times$, 14 mg kg⁻¹; $\bullet$, 30 mg kg⁻¹. *c*, Chromatin following methylmethanesulphonate (MMS) treatment. The solid line shows control chromatin from *a*; $\circ$, 50 mg kg⁻¹; $\times$, 200 mg kg⁻¹. Rat liver chromatin was prepared from liver nuclei by the procedure described²⁰ for thymus chromatin: the nuclei were prepared by a modification of the method of Widnell and Tata²¹. Methylating agents were dissolved in normal saline and injected intraperitoneally. *E. coli* DNA polymerase I was prepared by a slight modification of Jovin *et al.*²²: the enzyme used was the phosphocellulose fraction that had been further purified by ultra-centrifugation in a glycerol gradient and contained no endonuclease I nor exonuclease III: 0.1 units were added to each assay. Details of the assay have been given elsewhere². Varying amounts of chromatin and water (to give a final volume of 0.3 ml) were added and thoroughly mixed before incubation at 37° C for 30 min.

The template activity of control chromatin increased linearly with the amount of DNA (as chromatin) added to the assay system up to at least 20 μ g DNA per assay (see Fig. 1*a*). Under these conditions there is always an excess of enzyme over binding sites on the DNA present in the assay. Two factors preclude measurements of template activity at higher chromatin concentrations: (1) the difficulty of measuring small volumes of concentrated chromatin and (2) the large amount of precipitate since chromatin is insoluble in the assay mixture².

The template activity of chromatin from treated animals in all cases rises linearly with the amount of DNA (as chromatin) added to the assay mixture up to a certain level and is indistinguishable from controls; at higher DNA concentrations the template activity remains constant (see Fig. 1*b* and *c*). This maximum value depends, for any one sample of chromatin, upon the dose of methylating agent given, the time period between injecting the animals and preparing the chromatin, and the period of storage of the chromatin after preparation and before its assay. Figure 1 shows the dependence of incorporation of dTTP into acid-insoluble material, in 30 min, on the amount of chromatin added to the assay; in this experiment the chromatin was prepared 5 h after injection and was assayed as soon as possible (about 18 h after beginning preparation). Values are given for: (a) control chromatin, (b) chromatin prepared from DMN-treated animals, and (c) chromatin prepared from MMS-treated animals. Table 1 shows the variation of maximum template activity of the treated chromatin with dose and with period of storage of the chromatin at 4°.

Preliminary experiments (R. Saffhill, unpublished results) designed to measure the amount of DNA polymerase I that will bind to control and to treated chromatin using enzyme labelled with radioactive mercury⁸ (²⁰³Hg) have shown that chromatin from treated animals can bind at least 15 times more polymerase than control chromatin. Little, if any of this can be due to binding to chromatin protein. It has been shown that control chromatin does not bind DNA polymerase I inactively² since virtually all of the enzyme, apart from that bound actively to the chromatin is available, free in solution, for binding to poly (dAT)·poly (dAT). Further, irradiation of chromatin, which, like methylation, results in the formation of single-strand breaks, damaged bases, and apurinic sites does not produce an appreciable number of inactive enzyme binding sites². Finally, the ratio of the template activity (measured under conditions of template excess) of the DNA prepared from treated and control chromatin by centrifugation² in the presence of 2 M NaCl and *p*-aminosalicylic acid is similar to that obtained for chromatin itself, indicating that most or all of the inactive enzyme binding sites must lie in the DNA. Thus, *in vivo* treatment with methylating agents does in fact lead ultimately to the formation of single-strand breaks in the DNA that will bind DNA polymerase.

The single-strand breaks that are revealed in chromatin by DNA polymerase I are necessarily those in the regions of the DNA of the chromatin that are accessible to the enzyme. It has been shown² that only a small percentage of the DNA of chromatin is accessible to DNA polymerase and that this DNA lies within zones that are accessible to poly-L-lysine. Single-strand breaks are formed in other regions of the DNA but these would not be detectable in the present experiments. Thus, 'single-strand breaks' refer only to breaks in the regions of DNA in chromatin that are accessible to DNA polymerase I.

In vivo treatment with DMN and MMS leads (among other effects) to methylation of the chromatin: both the protein⁹ and the DNA¹⁰ are methylated. Very little is known about the methylation of the chromatin proteins apart from the fact that all of the histone fractions are methylated and the degree of methylation is considerably less than in the

TABLE 1 Effect of DMN and MMS on the template activity of rat liver chromatin

a, DMN						
Day of assay* (following preparation of chromatin)	2 mg kg ⁻¹		14 mg kg ⁻¹		30 mg kg ⁻¹	
	dTTP (pmol)†	Rel. no. of SSB‡	dTTP (pmol)†	Rel. no. of SSB‡	dTTP (pmol)†	Rel. no. of SSB‡
Day 1	5.2	7.1	3.9	9.5	2.3	16.1
Day 2	5.0	7.4	3.5	10.5	2.2	16.7
Day 3	4.7	7.9	3.3	11.2	2.1	17.5
b, MMS						
Day of assay* (following preparation of chromatin)	50 mg kg ⁻¹		200 mg kg ⁻¹			
	dTTP (pmol)†	Rel. No. of SSB‡	dTTP (pmol)†	Rel. No. of SSB‡		
Day 1	4.9	7.6	3.6	10.3		
Day 2	4.7	7.9	3.2	11.6		

* The chromatin was stored at 4°C.

† pmol dTTP incorporated in a 30 min incubation.

‡ The number of single-strand breaks (SSB) in control chromatin is taken as unity.

DNA (H. K. Cooper, thesis submitted). The sites of methylation in the DNA are the phosphodiester backbone¹¹ (producing very stable phosphotriesters¹² which are believed to be associated with the so-called X₁-products¹³ and the DNA bases¹⁴. The major methylated bases detected are 7-methylguanine, 3-methyladenine and 6-O-methylguanine, the latter being found only after treatment *in vivo* with DMN and not after MMS treatment¹³. The maximum 7-methylguanine content of the DNA is found 5 h after DMN treatment and 2-4 h after MMS treatment: at these times similar levels of 7-methylguanine are produced after injection of 2 mg DMN per kg and 50 mg MMS per kg. The methylated bases are lost from the DNA both *in vivo* and *in vitro* and their half-lives have been measured and found to be 3 d, 2-3 h (G. P. Margison, and P. J. O'Connor, personal communication) and 13 h respectively for the *in vivo* loss of 7-methylguanine, 3-methyladenine and 6-O-methylguanine. There is some evidence for the involvement of enzymes *in vivo*¹³ although this is presumably accompanied by some chemical depurination to yield apurinic sites in the case of the unstable alkylpurines. The nature of the excision and repair process is uncertain. It almost certainly involves the formation of a single-strand break at or near to a methylated base (or an apurinic site formed on loss of a base) at some stage followed by repair, and rejoining of the DNA. Thus, in the experiments reported here we are observing the single-strand breaks produced as part of such a repair process.

With excess enzyme, the template activity will increase linearly with the amount of DNA added to the assay, that is, with the number of active binding sites added. At lower chromatin concentrations the treated chromatin is indistinguishable from controls, which indicates that among the new enzyme binding sites produced as a result of the methylation, there are no detectable active binding sites. Inactive binding sites however, would, under these conditions of excess enzyme, merely bind an enzyme molecule that would otherwise be free in solution and not synthesising DNA, that is, there would be no detectable effect on the template activity¹.

Once the DNA concentration of treated chromatin is sufficient to provide an excess of enzyme binding sites, there is no further increase in template activity with DNA concentration. Thus the lowering of the maximum template activity (the value of which is not measurable in practice with control chromatin) arises from the formation of inactive enzyme binding sites following the action of the methylating agent.

The number of single-strand breaks in a sample of chromatin relative to the number in control chromatin can be determined from its template activity. The single-strand breaks produced *in vivo* following methylation of the DNA must all be inactive binding sites as their effect can be detected only under conditions of enzyme saturation (excess of template)¹. Under these conditions of enzyme saturation the

TABLE 2 Presence of single-strand breaks in the DNA of rat liver chromatin following *IN VIVO* treatment with DMN and MMS

Time after treatment	Relative number of single strand breaks*		
	14 mg kg ⁻¹ DMN	2mg kg ⁻¹ DMN	50 mg kg ⁻¹ MMS
Control	1.0	1.0	1.0
15 min	8.8	<4	<4
1 hour	16.1	12.6	—
2 h	17.6	9.7	7.1
5 h	9.5	7.1	7.6
12 h	—	—	10.9
24 h	7.4	5.2	14.7
72 h	4.6	—	8.4
168 h	<4	—	4.5

* The number of single-strand breaks in control chromatin is taken as unity.

template activity of a sample of chromatin is proportional to the fraction of all binding sites that are active in DNA synthesis. Although the maximum template activity for control chromatin cannot be directly measured, it can be estimated² that enzyme saturation would occur at about 80 µg DNA per assay and that the maximum template activity would be about 35 pmol dTTP incorporated in 30 min. Until sufficient single-strand breaks have been formed in the DNA to saturate the enzyme by 20 µg DNA per assay (the highest level used in the present experiments), no difference between control and treated chromatin is detectable. At 20 µg DNA per assay the condition of enzyme saturation is reached when the number of single-strand breaks has increased about 3 times. Thus, chromatin with a template activity indistinguishable from controls up to 20 µg DNA per assay is referred to here as having less than 4 times the number of single-strand breaks present in control chromatin.

The presence of single-strand breaks was investigated at different times following injection of DMN and MMS. This is shown in Table 2. The time course for two different doses of DMN (2 mg kg⁻¹ and 14 mg kg⁻¹) is similar, the number of single-strand breaks present in the DNA increasing very rapidly within the first few minutes after injection of the DMN and reaching a maximum after 1-2 h. In the case of MMS at 20 mg kg⁻¹ there is no rapid increase in the number of single-strand breaks in the DNA; instead, the increase is gradual and the maximum number is not reached until about 24 h after injection. This difference is very much greater than the error of the experiments.

The higher the dose, the more single-strand breaks are present in the chromatin 5 h after injection (see Table 1) but the increase is not linear with dose. One possible reason for this is that the degree of methylation is far in excess of the excision capacity of the cell which, presumably would be

working at maximum capacity at all doses.

The decrease in template activity (corresponding to an increase in the number of single-strand breaks in the DNA) of treated chromatin on storing could reflect some *in vitro* chemical depurination (loss of methylated purines) and subsequent hydrolysis of the apurinic sites to give inactive binding sites, certain methylated bases being known to be lost quite quickly from DNA *in vitro*¹⁵. The single-strand breaks arising from hydrolysis of apurinic sites by the proposed β -elimination¹⁶ would be expected to be inactive binding sites since they would lack the 3'-hydroxyl group that is necessary for DNA synthesis to take place³. Alternatively, the decrease could arise from the action of a contaminating exonuclease which acts upon methylated or apurinic DNA to give inactive enzyme binding sites: the enzyme being absent or not detectable in control chromatin which shows no change in template activity on storing.

In the experiments reported here we are observing the presence of single-strand breaks, within limited regions of the DNA (accessible to DNA polymerase I), which may be formed at several stages of the repair process. Other workers have followed the methyl groups remaining unexcised in the DNA^{13,15} or the single-strand breaks shown by alkaline sucrose gradient centrifugation^{17,18}, a procedure which reveals also potential single-strand breaks in the DNA (for example, apurinic sites which yield single-strand breaks under alkaline conditions).

We have shown here that within the first few minutes of DMN treatment there is a large increase in the number of single-strand breaks present in the DNA, indicating a very rapid methylation followed by a rapid removal of some of the methylated bases. The observed *in vivo* loss of 7-methylguanine cannot account for this although it is possible that the observed rates of removal of 3-methyladenine and 6-O-methylguanine could do so. Other possibilities are that other, more labile methylated bases are produced and are responsible, although these have not yet been detected (no studies have been made of the methylated base content of the DNA at very short times after injection), or that there is a very rapid enzymatic excision of some of the methylated bases from certain regions of the DNA.

The reason for the difference in the time course of single-strand breakage after DMN and MMS treatment is uncertain. It could be that the two agents methylate different regions of the DNA since they are chemically different in their mode of action: some, or all, of the regions methylated by DMN being more rapidly excised than those methylated by MMS. Another possibility is that MMS methylates and inactivates some of the enzymes involved in the repair process with a consequent delay in repair.

In summary, we have shown that methylation of DNA *in vivo* affects the template activity of chromatin for DNA polymerase I by producing single-strand breaks. A relatively large effect is detected after low doses of methylating agent and (in the case of DMN) at very short times after injection. Functional changes have not previously been found after very low doses of these agents. The results demonstrate also the usefulness of DNA polymerase I in the investigation single-strand breaks in DNA. Since the single-strand breaks produced as a result of the methylation are all inactive enzyme binding sites (they bind DNA polymerase but DNA synthesis does not take place), the 3'-hydroxyl group at the break must be blocked, presumably by a phosphate. This is indicative of the type of endonuclease acting upon the methylated and apurinic DNA. Recently endonucleases that specifically attack apurinic sites in DNA have been discovered¹⁹ but nothing is known of their specificity.

To determine the possible relevance of these results to carcinogenesis we intend to study the effect of mixture of DMN and MMS upon the template activity of chromatin prepared from various tissues, along with the effect of other

carcinogens.

We wish to thank Mrs J. M. Margison for injecting the animals. The work was supported by grants from the Medical Research Council and the Cancer Research Campaign.

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Received August 28; revised November 7, 1973.

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Detection and radioassay of soluble circulating immune complexes using guinea pig peritoneal exudate cells

METHODS at present available for the detection and quantitation of soluble circulating immune complexes include precipitation with C1q in agarose gel diffusion¹, precipitation with monoclonal rheumatoid factor², ultracentrifugation, inhibition of antibody-mediated lymphocyte cytotoxicity¹⁰, and precipitation with an optimal concentration of polyethylene glycol^{2,3}. These procedures either involve the use of substances isolated by fairly laborious means, or take some time to yield results. Preliminary tests in this laboratory showed that aggregated human immunoglobulin ingested by guinea pig macrophages was easily detectable by immunofluorescence, and that similar inclusions could be demonstrated after exposure of macrophages to systemic lupus erythematosus (SLE) sera.

We have used radioassay to measure uptake, and report here a new method applying the principle of competitive inhibition to the ability of guinea pig peritoneal exudate cells to ingest soluble immune complexes. The method takes only a few hours, and can detect as little as 25 ng of soluble immune complexes. Preliminary results showed the presence of complexes in twenty-two out of twenty-five randomly selected SLE sera (88%) and in none of twenty-five normal human sera. By the polyethylene glycol method, performed as described below, seventeen of the same twenty-five SLE sera

(68%) were positive for complexes as judged by the percentage of their total IgG precipitated.

Macrophages obtained about 5d after intraperitoneal injection of guinea pigs with sterile liquid paraffin were thoroughly washed in medium 199 three times by gently sucking up and down with a Pasteur pipette for 3 min and centrifuging at 500*g* for 7 min. The cells were then treated with human gamma globulin at a final concentration of 0%, and after another wash cellular debris was removed as described by Shortman *et al.*⁸. Human gamma globulin aggregated by heating at 63°C for 15 min was labelled with ¹²⁵I using the chloramine T method⁴ to produce labelled aggregate at a concentration of about 10 mg per ml.

Sera were tested for their inhibitory effect on uptake of labelled aggregate by the macrophages as follows. Tests were set up in triplicate in Falcon tubes containing 750,000 cells in 0.5 ml of medium 199 to which were added 10 μ l of a 1:5 dilution of serum in phosphate buffered saline (PBS), pH 7.4, and 10 μ l of the labelled aggregate solution diluted to give about 5000 c.p.s. The serum and aggregate were added simultaneously. In the control test, PBS was substituted for serum, in order to determine maximal uptake of labelled aggregate in the absence of competing serum. Cell counting was standardised by using a Coulter counter. All sera tested were decanted by incubating at 56°C

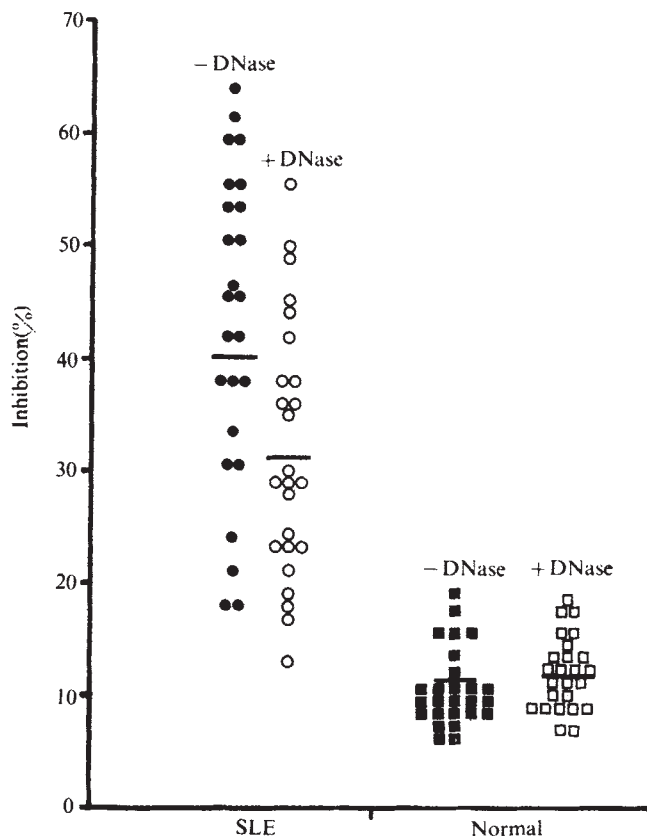


FIG. 1 Inhibition by human sera of uptake of labelled aggregated human gamma globulin by guinea pig macrophages. Percentage inhibitions and means for twenty-five SLE sera and twenty-five normal sera are shown under -DNase. Also shown is the effect of pretreatment of the sera with DNase. DNase I was added to the sera to a concentration of 50 μ g ml⁻¹, and the mixtures incubated at 37°C for 1 h in the presence of Mg²⁺. The mean inhibitory activity of the SLE sera was significantly ($P < 0.001$) reduced. As well as normal sera, sera from forty-one patients with diseases other than SLE were tested: osteoarthritis (10), ankylosing spondylitis (5), scleroderma (5), sarcoidosis (5), acute appendicitis (4), peritonitis (2), chronic bronchitis (5), carcinoma of the bronchus (2), carcinoma of the stomach (1), and carcinoma of the prostate (2). All gave inhibition within the normal range.

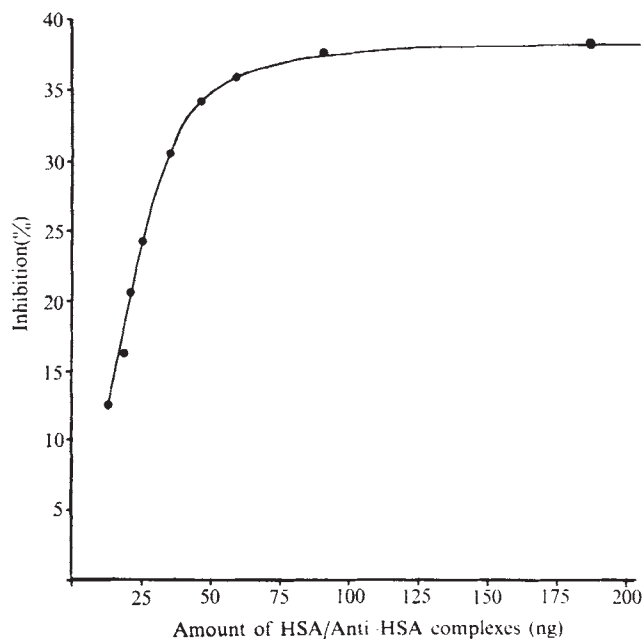


FIG. 2 Dose/response curve for inhibition of uptake of labelled aggregate by HSA/anti-HSA complexes. HSA/anti-HSA soluble complexes were prepared by determining the equivalent proportions and adding 50 times antigen excess to the antiserum. The precipitate forming after incubation was discarded and the supernatant containing the soluble complexes used in the test. The figures shown in the ordinate represent the protein content of various dilutions of the supernatant.

for 30 min, and centrifuged at 1000*g* for 20 min. Fresh sera, as well as sera frozen at -20°C could be used. After the addition of serum and aggregate, the tubes were incubated at 37°C for 75 min during which they were gently shaken about once every 15 min to ensure continuous and thorough mixing of the cells with the sera and aggregate. It is important to avoid mixing by inversion or the use of a rotator as this leads to loss of cells by adherence to the caps of the tubes. The cells were then washed carefully three times by centrifugation only (avoiding sucking up and down for the same reason), using medium 199 and making sure that the cells after each wash were not disturbed during the pipetting off of the supernatant. It was found best to leave a small volume of the supernatant over the pellet, and to resuspend the cells in it by a gentle shake before adding fresh washing medium. The final pellet comprising macrophages and their ingested materials was suspended in medium 199 and its radioactive content determined by counting in a Gamma Guard 150 (ICN Tracerlab).

Iodinated monomeric human gamma globulin diluted to give the same number of c.p.s. as the aggregated human gamma globulin was added to triplicate tubes treated in the same way, and the mean count was subtracted as blank.

Figure 1 shows the inhibition of uptake of labelled aggregate in the presence of competing serum, expressed as a percentage of the maximal uptake in the absence of competition. It can be seen that SLE sera exert a more pronounced inhibitory effect than normal sera. That the inhibitory effect of SLE sera is probably due to the presence of soluble immune complexes was demonstrated by an experiment in which a solution of artificially prepared complexes of human serum albumin (HSA), and rabbit anti-human serum albumin (anti-HSA), in 20% normal human serum (NHS), was substituted for SLE serum. Figure 2 shows the dose/response curve obtained by adding labelled aggregate in competition with various amounts of HSA/anti-HSA complexes in this way. The antiserum from which the complexes were prepared was added in place of PBS in the control test.

It was found that as little as 25 ng of complex exerts significant inhibition—an indication of the sensitivity of the method. With this graph as calibration, the amount of soluble complexes present in test sera is readily determined after making corrections for the dilution factor.

Polyethylene glycol (PEG) at suitable concentration precipitates soluble immune complexes. Precipitates obtained from five of the positive SLE sera by adding 0.5 ml of a solution of PEG in borate buffer, pH 8.5, to equal volumes of 1:25 dilutions of the test sera in borate buffer to reach a final concentration of PEG of 7.5% were recovered by centrifugation and redissolved in PBS. The resulting solution was inhibitory in the test whereas the supernatant serum after the addition of PEG showed no inhibition. This is additional evidence that soluble immune complexes are responsible for the inhibition.

Fractions were obtained by gel filtration of serum samples on columns of Sephadex G150, and their inhibitory activities were measured and compared with their IgG, IgM and IgA content as determined by radial immunodiffusion assay⁶. Figure 3 shows results with one SLE serum fractionated in this way; the peak of inhibitory activity occurred in fractions of larger molecular size than IgG. The fact that no IgG was detectable by the Mancini method in the earlier of these inhibitory fractions may be accounted for by the greater sensitivity of the inhibition test. It was further shown in this experiment that when the inhibitory fractions were precipitated with PEG at a final concentration of 7.5%, the resulting supernatants had lost their inhibitory activity.

In an experiment using DNase, we have shown that part of the inhibitory effect produced by SLE sera may be due to the presence of DNA/anti-DNA complexes by demonstrating a reduction in inhibition following DNase treatment. Figure 1 shows that treatment of twenty-five SLE sera with DNase reduced their mean inhibitory activity by about 25%, in agreement with the results obtained by Creighton² with the PEG method.

When complexes of HSA with ¹²⁵I-labelled rabbit anti-HSA prepared at various antigen-antibody ratios were added in competition with SLE serum in the inhibition test described above, we found (Fig. 4, shaded portion) that the relative inhibitory effect of SLE serum increased with increasing antigen excess in the competing artificial complex. As well as providing further confirmatory evidence that the inhibitory effect of this serum is due to the presence of immune complexes, this observation suggests a simple ap-

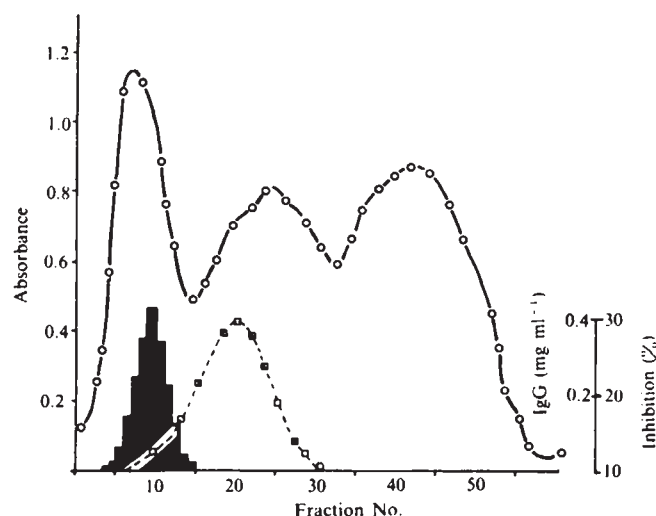


FIG. 3 Chromatography on Sephadex G150 of serum from one SLE patient showing the position of the inhibitory complexes. ○—○, Absorbance of fractions at 280 nm; □—□, IgG content of fractions; ■, inhibitory fractions.

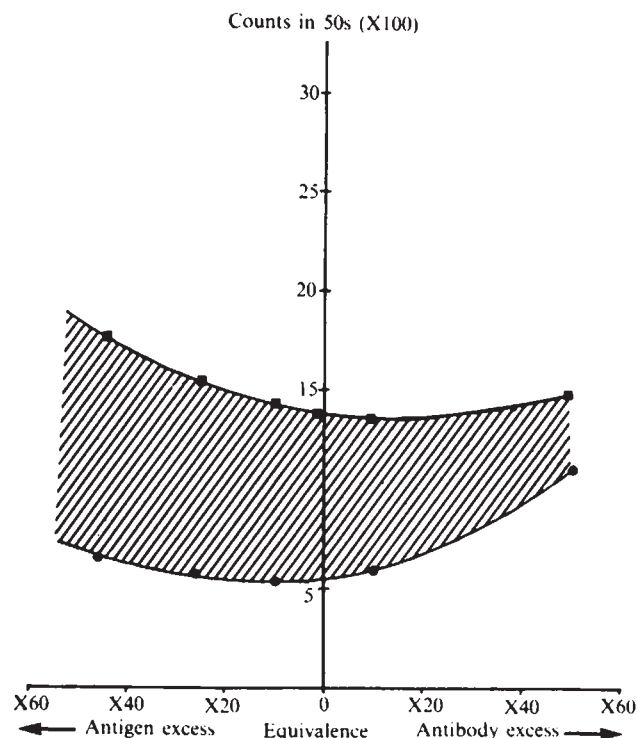


FIG. 4 The inhibitory effect of an SLE serum on the uptake of labelled HSA/anti-HSA by guinea pig macrophages. HSA/anti-HSA complexes were prepared by determining the equivalent proportions and adding 50 times, 25 times, 5 times, and 2 times antigen excess to a constant volume of antiserum. The precipitate forming after incubation was discarded and the supernatant containing the soluble complexes used in the test. ■—■, Counts in 50 s representing uptake of labelled complexes in the absence of competing serum; ●—●, counts in 50 s representing uptake of labelled complexes in the presence of SLE serum.

proach to the study of molar ratios in circulating soluble immune complexes.

Many factors may contribute to the inhibitory effect of sera on the phagocytosis of soluble labelled aggregate by guinea pig peritoneal exudate cells, including serum levels of IgG, serum complement levels, the presence of receptors for IgG and complement on the macrophage surface⁷, and the concentration of certain electrolytes, notably calcium⁸. For these reasons, the sera tested by this procedure were heat inactivated, and the cells were pretreated with human gamma globulin to 'block' surface receptors. The non-specific inhibitory effect of normal sera was thereby significantly reduced to a mean figure of 12.50% with 20% as the upper limit. The formation of soluble aggregates during heat inactivation may contribute to the inhibitory effect of sera, and very recently we have found that the use of heated sera in the test may be avoided by preliminary incubation of the cells with fresh guinea pig serum. This serves the dual purpose of 'blocking' both the complement receptors and at least some of the IgG receptors on the macrophages, and appears to obviate the need for pretreatment with foreign immunoglobulin.

The ability of foreign (guinea pig) macrophages to ingest soluble immune complexes present in human sera raises the question why human phagocytic cells do not *in vivo* by a similar process prevent such complexes persisting in the circulation, and eventually mediating tissue damage. One explanation might be that the level of circulating complexes in SLE can exceed the phagocytic capacity of blood monocytes and other phagocytes such as Kupffer cells. So far, however, we have been unable to obtain convincing evidence that human blood monocytes in SLE ingest soluble complexes *in vivo*. It is perhaps more likely that, although the

capacity of guinea pig macrophages to recognise the immunoglobulin moiety of complexes is inhibited by pretreatment with human immunoglobulin, the conditions of the test as carried out nevertheless increase the degree of denaturation of the antibody molecules in the complexes enough to enhance their ingestion.

We thank Mrs Ella Stephenson, Mr Paul Embling, and the staff of the Haematology Department, in particular Mrs C. Billing, for their assistance. We are grateful to Dr John Watkins, Dr Donald Rajapakse, and Dr Papamichail for their useful advice. One of us, I.I.O., is in receipt of a grant from the Commonwealth Medical Association for work leading to an MD thesis.

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Received August 22; revised October 2, 1973.

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Evidence against recessive inheritance of susceptibility to the chronic carrier state for hepatitis B antigen

THE discovery of Australia antigen (Au), now designated hepatitis B antigen (HBAG), and its association with type B viral hepatitis stimulated application of a variety of serological assays for the detection of HBAG and anti-HBAG in pretransfusion screening of donors' blood and epidemiological work relating to transmission of hepatitis¹⁻⁶. The reasons for chronic prevalence of HBAG in apparently healthy carriers, who form an epidemiological reservoir of hepatitis B virus (HBV), remain ill-defined. Based on family studies with HBAG, Blumberg *et al* proposed a hypothesis that the persistence of HBAG for long periods is evidence of a genetic susceptibility which is inherited as a simple recessive trait^{7,8}. The extensive family data, however, included no pedigree with progeny of two parents positive for the antigen. Such a family is crucial to lend validity to the genetical hypothesis. An independent study in Italy supported the hypothesis with certain qualifications⁹.

In reviewing the mass of published family data, Petrakis made the most critical observation that only one family in Sardinia had two parents positive for HBAG and only two of their seven children were positive for HBAG; this did not support the genetic hypothesis⁹. It is tenable however that the postulated inherited susceptibility would be shown only when people are exposed to the infectious agent, and it is conceivable that the remaining five children in the Sardinian family had not been exposed to HBV. A pedigree with two HBAG carrier parents and their progeny of three children

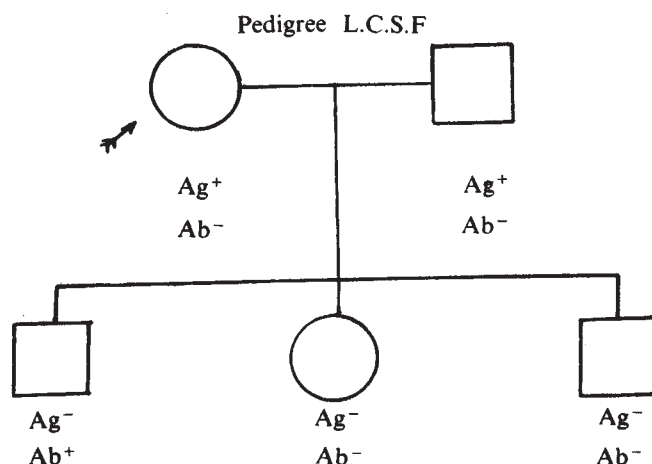


FIG. 1 A Chinese family with HBAG chronic carrier parents and a child with specific antibodies to HBAG. The other two children had neither HBAG nor anti-HBAG. Ag = HBAG; Ab = anti-HBAG.

were investigated for HBAG and anti-HBAG and the results presented in this report provide the first discrete evidence against the genetic hypothesis.

Tests for HBAG and anti-HBAG in the sera were performed by gel diffusion analyses, counterelectrophoresis and haemagglutination assays^{4,10}. Radioimmunoassay for HBAG in the sera negative by other methods was performed according to the instructions of the manufacturers of the kits (AUSRIA™, Abbott Laboratories, Chicago). The specificity of HBAG detected in any serum was confirmed by a line of identity with a known positive control serum in gel diffusion analysis. The specificity of the antibodies was confirmed by serological neutralisation of four units of antibodies with a known HBAG—positive serum and also by 10 μ g of purified HBAG¹¹. Serum samples from both parents were tested in the clinical chemistry laboratory for liver function tests.

The results of HBAG and anti-HBAG testing of each member of the pedigree are shown in Fig. 1. HBAG was consistently detected in the serum of both parents in repeated testing of their specimens obtained on three occasions over a period of six months. Both parents were in excellent health, had no apparent physical problems, and clinical or biochemical results suggested absence of liver abnormality. It was concluded that they were asymptomatic chronic carriers of HBAG¹². Both parents are immigrants from the People's Republic of China. The three children were negative for HBAG in repeated testing of their sera by every method including radioimmunoassay. The 10-yr-old son, however, had specific anti-HBAG with a titre of 256 in haemagglutination assay. The 5-yr-old daughter and 3-yr-old son had no antibodies. The absence of HBAG, but the detection of specific high titre anti-HBAG in the eldest child was considered unequivocal evidence of past exposure to HBAG. The fact that the boy failed to become a chronic carrier of HBAG but produced an immune response in the form of antibody production provides crucial evidence against the susceptibility for the chronic carrier state inherited as a simple autosomal recessive trait.

Tests for anti-HBAG in families similar to the one reported here may provide additional evidence against the genetic hypothesis. With an average incidence of three healthy HBAG-positive persons per 2,000 adults in the United States of America, the possibility of detecting such matings at random is small. The family clustering of HBAG-positive individuals is more likely a function of an increased opportunity for environmental exposure to the infectious agent than the recessively inherited susceptibility^{9,13}. These considerations may prove valuable in genetic counselling occa-

sionally sought by chronic carriers, particularly amongst informed professionals who are medically and epidemiologically more concerned about the infectivity of chronic carrier state.

This work was supported by a research grant from the Center for Disease Control and by a contract with the Blood Resource Branch of the National Heart and Lung Institute of the United States Public Health Service.

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Amino acid sequence change associated with genetic marker Inv(2) of human immunoglobulin

IMMUNOGLOBULINS are proteins composed of two heavy and two light chains covalently linked by interchain disulphide bonds¹. There are two types of light chains, κ and λ . The κ type human light chain is associated with an antigenic marker called Inv (ref. 2). Three types of antigens are recognised namely Inv (1), Inv(2) and Inv(3). Ropartz, Rivat and Rousseau³ have shown that these Inv markers are inherited through a series of three alleles *Inv*^{1,2}, *Inv*¹ and *Inv*³. About 98% of Caucasoid individuals who have the Inv(1) antigen also have the Inv(2) antigen³. Since fewer than 20% Caucasoids have the Inv(1, -2) phenotype⁴ it follows that the *Inv*¹ allele is rare in this race.

The Inv markers are associated with the presence of leucine and valine in position 191. Residue 191 is leucine in the case of Inv (1, 2) and valine in the case of Inv(3) in monoclonal κ chains^{5,6} and in normal polyclonal light chains⁷. The primary sequence associated with the third antigen, Inv (1, -2), has remained unknown.

We now report studies of a κ -Bence Jones protein, Cro, which is Inv(1, -2, -3). Figure 1 shows the Inv phenotype of the kindred of the patient. It seems reasonable to assume that an *Inv*¹ allele is present in this family and that the failure to detect Inv(2) in the Bence Jones protein is not an artefact caused by the patient's disease.

Protein Cro was purified, reduced, carboxymethylated and digested with trypsin. All the tryptic peptides of the C region

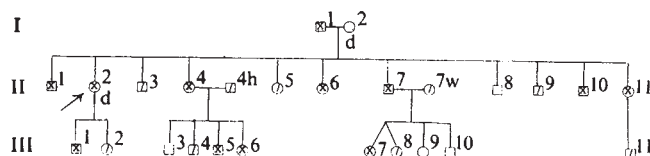


Fig. 1 Pedigree of the Cro family. X = Phenotype Inv (1, -2, 3). / = Phenotype (-1, -2, 3). Blank = not tested.

were isolated. They all had an amino acid composition identical to the corresponding homologous tryptic peptides of a κ chain except for peptide TIar. The latter peptide could be ascribed to position 150-169 by homology but did not contain alanine and it had an extra valine. The N-terminal sequence of peptide TIar was determined using the combined procedure of dansylation for N-terminal analyses and Edman degradation⁸. The result obtained was

Val-Asx-Asx-Val-Leu (Glx₅, Ser₄, Gly, Asx₂, Val, Thr) Lys

The fourth position is occupied by alanine in all the other known κ chains.

Peptide TIInBR (position 191-207) contained two leucines and two valines in its composition indicating that position 191 was leucine. In addition one of us (A.G.S.) isolated from the aminoethylated κ chain a tryptic peptide with an amino acid composition (Leu, Tyr, Ala)Cys which by homology with known chains can be situated in position 191-194.

The relation between Inv phenotype and primary structure of the C region of the κ chain is therefore as shown in Table 1. The last combination of residues has not been observed and may not exist since it seems likely that the allele Val₁₅₃Leu₁₉₁ evolved from the gene Ala₁₅₃Leu₁₉₁. Alternatively, it may correspond to the Inv(-1, -2, -3) serum reported by Steinberg *et al.*⁹.

TABLE 1 Inv phenotype and K chain structure

Inv	Residue	
	153	191
1, 2, -3	Ala	Leu
-1, -2, 3	Ala	Val
1, -2, -3	Val	Leu
?	Val	Val

All primates tested by van Loghem¹⁰ were Inv(-1, -2). Alepa and Terry¹¹ found polymorphism for both Inv(1) and Inv(3) in chimpanzees. Inv(1) has been found in baboons¹². Neither Inv(1) nor Inv(2) has been found in other monkeys. At present it is difficult to assert the evolutionary relationship between these different genes.

No allotypes in human λ chains have been described but isotopic changes have been reported in protein Kern at position 153 (ref. 13) and in the Oz locus at position 190 (ref. 14). These changes are under the control of non-allelic structural genes. The striking similarity in the location of the changes in the C region of the λ and κ chains probably means that these positions are more likely to undergo changes. Poljak *et al.*¹⁵ and Schiffer *et al.*¹⁶ have built crystallographic models of a Fab' fragment of a human immunoglobulin, New, and a Bence Jones protein respectively. In both cases it has been observed that both positions 152 and 190 occur very loosely in space. The light chain of both proteins is of the λ type but it seems likely that the tertiary structure of κ chains will be very similar if not identical to that of the λ chains. From a 2Å crystallographic model (R. J. Poljak, personal communication) of the Fab' fragment of protein New it is possible to see that the positions of the λ chain, equivalent to

153 and 191 in κ chains are located on the surface of adjacent loops, exposed and at a distance of less than 10Å. Furthermore the orientation of the residues is such as to render them accessible to solvent and reagents. Our data indicate that the antiserum Inv(2) recognises leucine at position 191 encompassing position 153. The crystallographic data makes this interpretation acceptable. When position 153, alanine, is replaced by a bulkier amino acid, valine, the Inv(2) reagent is sterically hindered and cannot recognise leucine 191. Antiserum Inv(1) probably does not encompass position 153 and therefore does not distinguish the valine/alanine substitution.

C.P.M. thanks Mr E. V. Deverson for technical assistance. A.G.S., C.L.M. and A.S. acknowledge US Public Health Service Research Grants from the National Institute of General Medical Sciences and the National Cancer Institute respectively and a research grant from the American Cancer Society

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Effect of 5-fluorouracil on the gibberellic acid-induced formation of thermolabile α -amylase molecules in barley aleurone cells

IN the aleurone cells of barley seed gibberellic acid (GA) stimulates *de novo* synthesis of α -amylase^{1,2}. From a study of the effect of 5-fluorouracil (FU) on the GA-evoked formation of thermolabile α -amylase molecules, Carlson³ has concluded that GA regulates a post-transcriptional control point. According to my interpretation, which differs from Carlson's conclusion, his data (Fig. 1, ref. 3) suggest that mRNAs for α -amylase are formed in the presence of GA.

Results of studies⁴⁻⁷ of nucleic acid metabolism are consistent with the concept¹ that the formation of α -amylase depends on specific RNA synthesis. Zwar and Jacobsen⁷ confirmed the finding⁵ that messenger-like RNAs are synthesised in tissue treated with GA. Other results suggest a close correlation of the synthesis of α -amylase with this effect of GA, as well as with modifications in RNA synthesis⁸ and the conversion of monosomes to polysomes⁹. Unlike actinomycin D, fluorouracil does not affect the total amount of α -amylase produced by aleurone cells incubated with GA⁷; it does not inhibit the formation of mRNAs⁷, nor does it affect the percentage of polysomes⁹ in the cells. The observed⁷ inhibition of rRNA and tRNA synthesis in aleurone cells is consistent with similar inhibitory effects of the analogue in other tissue¹⁰.

Jacobsen *et al.*¹¹ found that α -amylase produced by GA-treated aleurone cells consist of at least four isoenzymes: 3 and 4 are rendered inactive, while 1 and 2 are stable to treatment with EDTA and sodium metahexaphosphate. Isoenzymes 1 and 2 represent 20-25% total α -amylase activity. Calcium is an integral part of all four isoenzymes and 1 and 2 are thought to bind Ca^{2+} very strongly. Carlson³ found that when Ca^{2+} was removed from purified α -amylase (presumably from isoenzymes 3 and 4 only) the thermolability (denaturation at 72° C followed by renaturation with addition of Ca^{2+}) of the enzyme formed in the presence of FU was greater than that of the 'native' (without FU) enzyme. The thermolability curves (Fig. 1, ref. 3) show that the percentage of thermolabile α -amylase molecules in the purified preparations either increased or decreased depending on whether the tissue was incubated with FU before or after the addition of GA. For example, the data (Fig. 1, ref. 3) obtained when the preparations were subjected to 72° C for 15 min show that 91% of the enzyme molecules produced by the tissue treated with FU for 72 h were thermolabile (Table 1b). Under identical conditions of denaturation 60% of the native enzyme (Table 1a) was also thermolabile. Carlson³ assumed that the thermolability of the α -amylase resulted from a misreading of the FU-containing RNA templates. Consequently, the enzyme preparations were expected to contain, in addition to normal molecules, thermolabile molecules formed on FU:RNA templates. Therefore, the change in the time (min) required to inactivate 50% of the enzyme (T50%) is also a measure of the increase or decrease in thermolabile enzyme molecules in the preparations. Table 1 shows that the T50% of the native α -amylase molecules decreased from 12 to 5 min when the enzyme molecules were formed by tissue incubated with FU continuously for 72 h. These findings raise the question of whether FU:RNA templates for synthesis of α -amylase are formed before or after addition of GA.

Since the tissue treated with FU for 72 h produced 91% labile enzymes, 83-85% of the molecules were formed on FU:RNA templates made by the tissue incubated with GA and FU together for 24 h (Table 1d and f). The data from the reciprocal experiment (Table 1c and e), in which the tissues preincubated (no GA) with 10^{-4} FU for 24 and

32 h respectively, produced 70 and 76% thermolabile α -amylase molecules, suggests that the FU:RNA templates were formed before the addition of GA. These tissues were preincubated in the presence of 2.5×10^{-4} uracil, which decreased the percentage of thermolabile molecules from 83 to 76% in 24 h and from 85 to 70% in 32 h. An 8-h pulse of uracil given to a tissue preincubated with FU for 24 h dilutes the pool of analogue (U:FU ratio increases from 4.7 to 77.0) and increases the FU-containing acid-insoluble RNA species³.

TABLE 1 Effect of 5-fluorouracil on percentage of thermolabile α -amylase molecules produced by GA-treated aleurone cells

Treatments	Thermolabile enzyme (%)	T50%
(a) Control (no FU)	60	12.0
(b) FU present for 48 h before and during the 24 h treatment with GA	91	5.0
(c) FU present for 24 h before, U present during the 24 h treatment with GA	76	5.5
(d) FU present for 24 h with GA	83	5.0
(e) FU present from 32 to 8 h before GA application, U present during 24 h treatment with GA	70	8.0
(f) FU present for 8 h before, and during 24 h treatment with GA	85	5.0

The percentage of enzyme activity that was stable on heating the EDTA-sodium metahexaphosphate-treated enzyme preparations at 72°C for 15 min was obtained from Fig. 1, ref 3. The T50% value, obtained by line intercept of the thermolability curves (Fig. 1, ref. 3) represents the approximate time (min) required to inactivate 50% of the enzyme activity at 72°C. Experimental details are described in ref. 3.

Does the increase in FU-containing RNA species represent the formation of FU:RNA templates? The mRNA is a very small percentage (about 1%) of the total cellular RNA⁷. Although FU inhibits (about 50%) the synthesis of ribosomal and tRNA, it has no effect on the formation of mRNAs or on the total α -amylase produced by the GA-treated cells⁷. α -Amylase is only one of many hydrolases produced by the aleurone cells treated with GA. Time course studies have revealed an actinomycin D-sensitive phase before the formation of α -amylase^{1,12} and protease¹³. The decrease in the percentage lability (from 85 to 70%) and the increase in the T50% value (from 5 to 8 min) resulting from the addition of uracil therefore suggests that enzyme molecules similar in properties to the native enzymes (thermolability 60%, T50% 12 min) are formed in the presence of uracil and after the addition of GA. Since the tissue incubated with FU and GA together for 24 h produced 83% thermolabile molecules compared with 91% in the controls (FU for 72 h), the 'logical' conclusion is that specific FU:RNA templates for the synthesis of thermolabile α -amylase molecules are formed after addition of GA.

In the absence of statistical data (Fig. 1, ref. 3), the differences in the percentage thermolability of the different enzyme preparations (Table 1) are difficult to assess. The decrease in the time required to inactivate thermally 50% of the enzyme molecules as a result of incubation with FU and GA together (Table 1b, d and f), suggests that the FU-containing RNA templates for the synthesis of thermolabile α -amylase molecules are formed after addition of GA. The validity of such a conclusion is based on the assumption that FU:RNA templates allow for the formation of thermolabile enzymes which are otherwise identical in isoenzyme composition, Ca²⁺ binding and other properties to the native molecules. Carlson's discovery⁸ that FU allows the formation of thermolabile α -amylase molecules, and the finding⁸ that

alterations in nucleic acid methylation prevent enzyme formation, support the suggestion¹⁴ that in the aleurone cells the action of GA involves specific structural modifications of the transcribed substance(s) (RNAs), and consequently a translational modulation of protein synthesis.

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Received June 18; revised December 21, 1973.

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Intensity Fluctuation Spectroscopy of Motile Microorganisms

LIGHT microscopy indicates that in *Escherichia coli* translational motility ('running') is accompanied by considerable side-to-side or wobble motion. Moreover, in the case of chemotactic strains Berg and Brown¹ showed that a typical bacterium spends considerable time in a 'twiddling' state in which translation ceases in favour of jittering rotational motion. In both the running and twiddling state the amplitude of the non-translational component of motion is comparable with λ , the wavelength of light. It is reasonable to expect therefore that the dynamics of both wobble and twiddle motion can be studied by intensity fluctuation spectroscopy² (IFS) of scattered laser light. We have examined the effects of wobble motion on the time dependence of the fluctuating light intensity scattered by a non-chemotactic mutant of *E. coli* whose motion is dominated by running with almost no twiddling. We conclude that IFS is sensitive primarily to the rotational component of the motion.

Nossal³ has demonstrated theoretically that under certain conditions IFS is sensitive to the distribution of translational swimming speeds for motile microorganisms. These conditions are: (a) that the particles swim at a constant speed for distances not less than λ ; (b) that the amplitude of non-translational motion is small compared with λ , and (c) that the particles are small compared with λ and (or) spherically symmetric. Although Nossal *et al.*⁴ presented experimental evidence that these assumptions are met in wild type *E. coli*, Schaefer⁵ was unable to reproduce these results, attributing the discrepancies to wobble motion superimposed on translation. Nossal and Chen⁶ subsequently studied the dependence

of motility of *E. coli* on chemoattractant concentration. They assumed that reasonably accurate mean-square translational speeds can be obtained from the initial decay of the intensity correlation function of the scattered light intensity.

In this article we establish the importance of rotational motion by two lines of evidence. First, we discuss a theoretical model in which motility is modelled as a form of uniform helical motion. This is applied to IFS data obtained on a non-chemotactic mutant of *E. coli* (*cheC497*) whose motion seems to be uniformly helical under most conditions (this type of helical motion would appear as side-to-side wobble under a microscope). Although the model is too restrictive for quantitative application, it establishes that IFS is relatively insensitive to the translation swimming speed of *E. coli*. This has been confirmed under viscosity conditions such that wobble motion is suppressed.

A bacterium is considered here to be rod-shaped with length b and negligible width. The body is presumed to simultaneously spin and translate, the body fixed at an angle α with respect to the direction of translation (x axis in Fig. 1). The

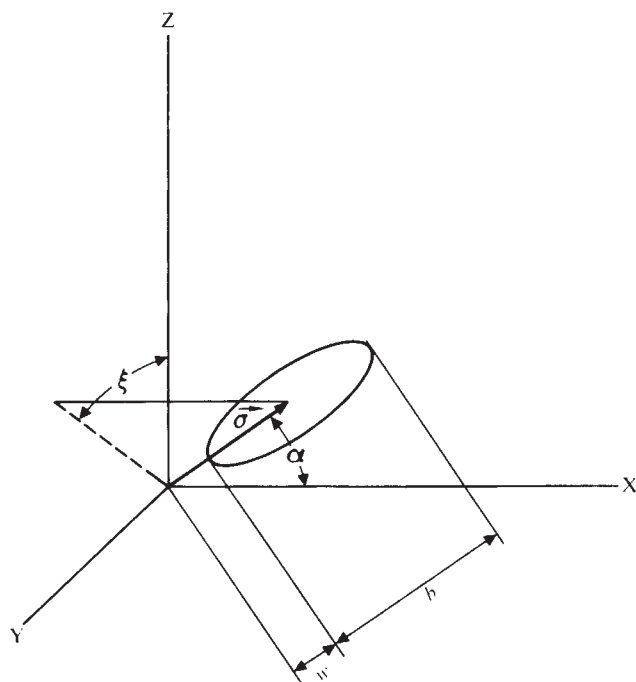


Fig. 1 Coordinate system used in model calculations.

body rotates about the translation direction with a single frequency Ω ; $\xi(t) = \Omega t$, where t is time. The intersection of the body axis (σ) and the translation axis is a distance w from the posterior (or equivalently anterior) end of the bacterium.

We make two important assumptions. First, rotational and translational motion are uncorrelated. Although this is not so for helical motion, expansion of the intensity correlation function about $t=0$ indicates that the result obtained below is similar to that expected for correlated motion. Second, to retain tractable equations it is necessary to neglect interference of light scattered from different parts of the same particle. This assumption is sufficiently restrictive that, for asymmetric bacteria, the results can only be used to demonstrate the relative importance of rotational and translational motion.

Within the framework of our model, $R_E(t)$, the correlation function of the scattered light field, takes the following form:

$$R_E(t) = \langle \exp\{i\mathbf{K} \cdot [\mathbf{R}(t) - \mathbf{R}(0)]\} \rangle \left[\frac{1}{b} \int_{-b/2}^{b/2} d\sigma \langle \exp\{i\mathbf{K} \cdot [\boldsymbol{\sigma}(t) - \boldsymbol{\sigma}(0)]\} \rangle \right] \quad (1)$$

The corner bracket indicates an ensemble average. For incident light of wave vector of magnitude K_0 , $K = 2K_0 \sin \Theta/2$, where Θ is the scattering angle. $\mathbf{R}$ is the position of the intersection of the body axis and the translation axis and $\boldsymbol{\sigma}$ is the position of the scattering element relative to $\mathbf{R}$.

If angular and translational motion is uniform for distances large compared with K^{-1} then equation (1) reduces to

$$R_E(t) = [1/4\pi b] \int_{-b/2}^{b/2} d\sigma \int_0^{2\pi} d\phi \int_0^\pi d\theta \sin \theta \exp[2iK\sigma \sin \phi \sin \theta \sin \alpha \sin(\Omega t/2)] \times \int_0^\infty dV_x [\sin(KV_x t)/KV_x t] P(V_x) \quad (2)$$

where θ and ϕ are the polar and azimuthal angles of $\mathbf{K}$ with respect to the x axis. $P(V_x)$ is the swimming speed distribution, V_x being the projection of the centre-of-mass velocity on the translation axis. For $t \ll \Omega^{-1}$,

$$R_E(t) = \frac{Si[K(b+w)\Omega t \sin \alpha] - Si[Kw\Omega t \sin \alpha]}{Kb\Omega t \sin \alpha} \times \frac{Si(KV_m t)}{KV_m t} \quad (3)$$

where Si is the tabulated sine integral⁷. In deriving equation (3) $P(V_x)$ has been assumed to be uniform up to a maximum speed V_m (the final results are insensitive to this distribution). Since $(b+w)\Omega \sin \alpha$ is the speed of the tip of the spinning organism, the rotation factor of equation (3) will be important whenever the tip speed is comparable with or greater than V_m .

Several observations are pertinent to equation (3). First, measured correlation functions should scale when plotted as a function of Kt if the assumptions of our model are met. Second, breakdown of Kt scaling indicates either high rotational frequencies, nonuniform motion over distances of the order of K^{-1} , or possibly particle asymmetry. Third, $R_i(K, t)$, the experimentally measured photocurrent correlation function, is proportional to the intensity correlation function rather than the field correlation function. The time-dependent part of $R_i(K, t)$, however, is proportional to $|R_E(t)|^2$ so that experimental data are compared with the square of equation (3).

Our experimental work involved measurement of the $R_i(K, t)$ for light scattered from a non-chemotactic strain of *E. coli* (*cheC497*)^{1,8}. The bacteria were studied under viscosity conditions conducive to both normal motility (showing significant non-translational motion) and motility in which the amplitude of off-axis motion was suppressed. In either case, motility in *cheC497* is dominated by running with little twiddling¹.

Samples were grown and studied in closed tubes containing Hutner's medium + 0.5% dextrose⁹. Samples were grown initially at 22° C and then at 30° C for at least three generations. All results were obtained at 30° C during the stationary phase, when the decay time of $R_i(K, t)$ showed a broad (1–3 h) minimum. We used high density populations (absorbance ~ 0.5) to avoid complications due to fluctuations in the total number of bacteria in the scattering volume^{5,10}. The incident wavelength was 6,328 Å and the experiments were performed with a SAICOR 43A correlator. A dilution experiment was performed as a control. A typical sample was diluted by a factor of four with air-saturated distilled water. No immediate change was observed in $R_i(K, t)$, suggesting that neither interparticle interactions nor small changes in oxygen concentration significantly affected our results.

The bacteria were also observed by light microscopy. Wobble motion with $\langle \alpha \rangle \sim 35^\circ$ was observed during all phases of growth (except in solutions containing Methocel). We tried to determine $\langle \Omega \rangle$ using stroboscopic illumination. Although high translational speeds precluded accurate measurement of $\langle \Omega \rangle$, angular velocities exceeding 10^2 rad s^{-1} were observed for certain individuals.

Figure 2 shows $R_i(K, t)$, the measured photocurrent correlation function, at several scattering angles for samples in culture medium (viscosity = 0.82 cP at 30° C). The very short characteristic times of these curves indicate that the decay

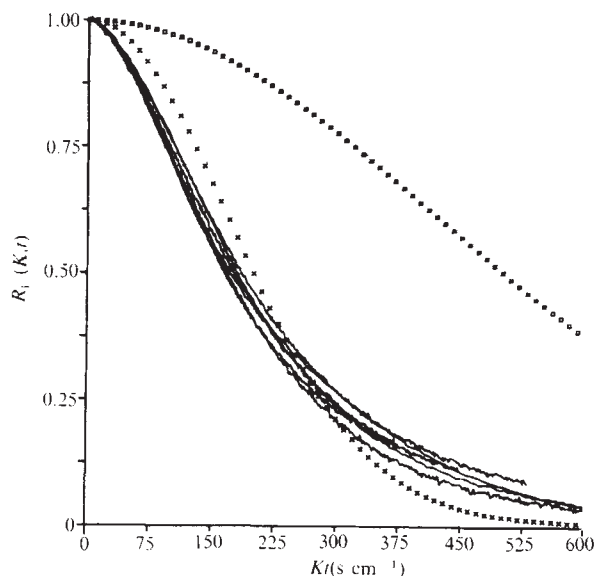


Fig. 2 Measured photocurrent correlation for light scattered from *E. coli cheC497* at $\Theta=20^\circ, 35^\circ, 50^\circ, 70^\circ, 90^\circ$ (solid lines) compared with calculated curves for pure translation (squares, $\alpha=0$, $V_m=50 \mu\text{m s}^{-1}$) and helical motion (crosses, $\alpha=35^\circ$, $b=2.7 \mu\text{m}$, $w=0.3 \mu\text{m}$, $\Omega=60 \text{ rad s}^{-1}$, $V_m=50 \mu\text{m s}^{-1}$).

was not caused by translational motion since analysis based on a pure translational model ($\alpha=0$) yields average speeds of more than $100 \mu\text{m s}^{-1}$, which are unreasonably large. Also, Kt scaling predicted by equation (3) was observed. This scaling was not observed for scattering angles larger than 90° or for other strains of *E. coli*.

Two theoretical curves are presented in Fig. 2. The squares represent the square of equation (3) assuming rectilinear motion ($\alpha=0$) and the crosses are calculated for helical motion with $b=2.7 \mu\text{m}$, $w=0.3 \mu\text{m}$, $\alpha=35^\circ$, $\Omega=60 \text{ rad s}^{-1}$ and $V_m=50 \mu\text{m s}^{-1}$. Although our theory is not expected to predict the curvature, a comparison of the characteristic times of these curves indicates the dominance of non-translational motion. The significance of Kt scaling displayed by these data cannot be established by reference to equation (3), due to the neglect of intraparticle interference.

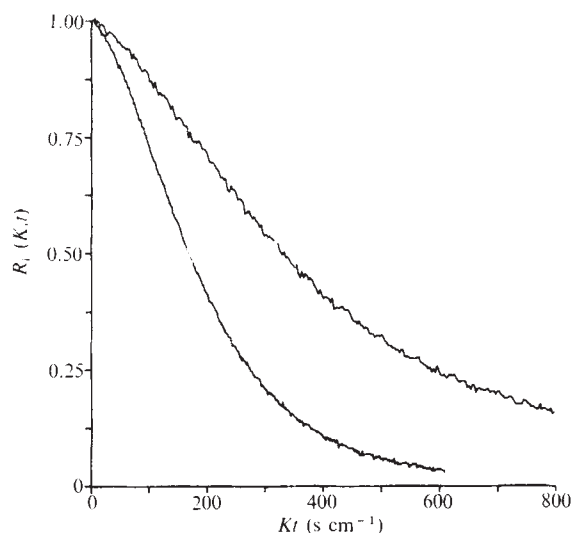


Fig. 3 Comparison of photocurrent correlation function of light scattered from *E. coli cheC497* at different solution viscosities. Data were taken at 30°C with $\Theta=70^\circ$. Upper curve, 1.6 cP; lower curve, 0.8 cP.

The most conclusive evidence for the importance of rotational motion was established by comparison of data taken from different solution viscosities. Figure 3 shows $R_t(K, t)$ for light scattered at 70° scattering angle from samples grown in the culture medium and in this medium plus 0.2% hydroxypropyl methyl cellulose (Dow Methocel 90 HG, viscosity 1.6 cP at 30°C). In the latter medium the translational speed is 20% greater, and the wobble motion is almost completely suppressed¹¹. Thus, if translation were dominating the decay of $R_t(K, t)$ a shorter characteristic time would be observed in 0.2% Methocel. In fact, a considerably longer characteristic time is found consistent with the decreased amplitude of off-axis motion ($\langle \alpha \rangle$ smaller). This establishes the dominance of non-translational motion at low viscosities. Considerable variability ($\pm 20\%$), was observed for the decay times in 0.2% Methocel, making it difficult to establish whether Kt scaling was observed.

Our results contrast with those obtained by Nossal *et al.*^{4,6} who were able to grow non-rotating cultures. We were unable successfully to repeat their experiments because (a) cultures showed great variability when collected in the log phase; (b) centrifugation led to greatly increased correlation times due to non-swimming bacteria; (c) number fluctuations¹⁰ often obscured the results at low population densities, and (d) cultures grown below 30°C had noticeable numbers of non-swimming bacteria.

We conclude that in most cases IFS is not directly sensitive to the translational speed of motile microorganisms such as *E. coli*. Although this diminishes the utility of IFS for study of centre-of-mass motion, it facilitates study of rotational motion, which is important in the understanding of flagellar action¹¹ and chemotaxis^{1,12}. Furthermore, since rotational frequencies are likely to scale with translation speed for a given solution viscosity, IFS can still be used to study the effects of environmental parameters (temperature, chemo-attractant concentration⁶) on swimming speeds.

We thank H. C. Berg for this mutant. We also thank him and R. A. Anderson for discussions. We thank J. P. Boon, R. Nossal and S.-H. Chen for communicating an unpublished manuscript. This research was supported by the US Atomic Energy Commission and by a grant from the National Science Foundation.

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Received September 21; revised November 12, 1973.

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Is craniofacial asymmetry and adaptation for masticatory function an evolutionary process?

MANY workers have investigated asymmetry of primate skulls. Woo¹ carried out direct chordal and arcual measurement on a large number of human skulls from the 26th to the 30th Egyptian dynasties. He found the bones of the cranium exhibited an asymmetry with the right side being larger, reflecting the development of the right hemisphere of the brain. The contralateral side of the facial complex exhibited an asymmetry with the left zygoma and left maxilla being larger. The lower third of the face was not investigated.

Mulick² investigated human facial asymmetry using cephalometric skull radiographs with a three-dimensional grid system of analysis and reported a facial asymmetry in six same-sex triplets, with the larger side being to the left.

Groves and Humphrey³ reported an asymmetry of gorilla skulls (*Gorilla gorilla beringei*) with the left side exhibiting a marked increase in length from the temporal fossa to the gnathion. They postulated that such asymmetry may be consequent to an asymmetry of function of the masticatory system.

The skull complex consists of numerous constituent parts. It is, therefore, the degree of harmony between the parts which determines the symmetry of the whole. The following investigation into human facial asymmetry was devised to establish a method for analysis of overall facial asymmetry in terms of its component parts, each of which can individually vary between the right and left sides.

Sixty posterior-anterior cephalometric skull radiographs of normal children were traced. No child with a degree of clinically evident or unacceptable facial asymmetry or gross deviation of dental arrangements was included.

Six bilateral and four single roentgenographic landmarks were delineated. The single landmarks were: sella; anterior nasal spine; incisal point; menton. The bilateral landmarks were: orbitale; centre of condylar shadow; zygomatic point; upper molar point; gonion; superior extent of condyle.

To assess the relative asymmetry of the component parts of the facial complex, a method of triangulation was used. The roentgenographic landmarks were joined to form triangles on both sides of the midline, representing the right and left mandibular regions, lower, middle and upper maxillary areas and the cranial base regions. The sides of the triangles were measured to the nearest 0.5 mm. and the areas compared with the areas of the equivalent triangles of the contralateral side.

The investigation revealed an overall asymmetry in most cases with the larger side to the left. The cranial base region, lower maxillary region and mandibular region exhibit a left sided excess. The maxillary region showed a right sided excess and the dento-alveolar region the greatest degree of symmetry.

The findings are of interest in that they suggest a compensatory adaptation during growth to effect an integration of the facial components. Scott⁴ suggested that the facial skeleton should be considered as a unit built up of a number of semi-independent regions, each with its own pattern of growth and development. The orbits, nasal cavities and lower border of the mandible show a high degree of independence and are under genetic control in their determination with the dento-alveolar region and lower parts of the nasal cavities showing a greater response to functional variation. These suggestions may be supported by the present findings. The mechanism whereby this occurs may be part of an evolutionary process. Adaptation of the dento-alveolar structures to muscle pressures is well recognised.

It is reasonable to assume that optimal function is provided by maximum cuspal interdigitation of teeth. We accept that this relationship can be arrived at in occlusion (that is, with

the teeth together) even though facial asymmetry may still exist. If in the rest position, or in the habitual postural position of the mandible, the upper and lower teeth are not co-incident about the sagittal plane, then an asymmetrical functional activity of both temporomandibular joint mechanisms must compensate during chewing and non-chewing activities in which the teeth are approximated. This in clinical practice is frequently related to pain and dysfunction and is therefore not a normal adaptation in humans.

To enable bilaterally symmetrical function and maximum intercuspation of the teeth to occur, compensatory changes seem to be operating in man in the growth and development of the dento-alveolar structures which minimise the underlying asymmetry in the spatial arrangement and size of the jaws.

This factor whilst no longer being essential for man's survival with his modern diet may nevertheless be regarded as a possible factor in the evolution and natural selection processes of the subhuman species.

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Received August 7, 1973.

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Deltatheridium and Marsupials

THE evidence for the radical transfer of the famous genus *Deltatheridium* to the Marsupialia seems to be partly different from that already presented¹. The cheek tooth formula, a key character separating marsupials and placentals, is questionable for *Deltatheridium*. There are seven cheek teeth, as usual in both groups, and the fourth is molariform, as in marsupials. This is evidence on phyletic affinity only if the primitive state, that of the latest common ancestor, was otherwise. In a manuscript that has circulated privately since 1963 I have argued from diverse evidence that the seven cheek teeth of each group may well be directly homologous with those of the other, with an ambiguity as to the permanent or deciduous premolars. In other words, P₄¹ or DP₄¹ of placentals may well be homologous to M₁¹ of marsupials. If so, a more or less molariform state of the fourth cheek tooth is primitive to both groups and the often nonmolariform state in more or less primitive placentals is secondary. This was why I suggested² that *Deltatheridium* might have one more molar than previously thought; the suggestion is now confirmed. Relative wear of the teeth is a useful but unreliable criterion (ref. 3, footnote on page 86).

Positive evidence, however, comes from the fact that there is a sharp morphological break between the third and fourth cheek teeth. This is characteristic of marsupials but not of primitive placentals or the pantothere quasi-ancestor of both groups, *Peramus*⁴.

If *Deltatheridium* is a marsupial, as seems entirely possible, it is specially similar only to the Stagodontidae^{5,6} among possible relatives. Butler and Kielan-Jaworowska ascribe this similarity to parallelism, but there seems no positive evidence of such. Normal marsupial styler cusps are retained in *Didelphodon* but not *Deltatheridium*, so some divergence is indicated. I prefer to classify the ancestors of marsupials and placentals as marsupials, which increases the probability of allocation of *Deltatheridium* to marsupials without affecting the phyletic evidence.

It should be noted that some time ago⁷ I abandoned my ordinal name, *Deltatheridia*⁸ (I now use *Hyaenodonta*), without change in the phyletic and adaptive evidence^{3,9,10} used to establish the order. Dr Kielan-Jaworowska showed me her new material on a recent visit but my comments are based on the published data.

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Temperature-sensitive mutations affecting the regenerative sodium channel in *Drosophila melanogaster*

In an attempt to elucidate the functions of the nervous system using behavioural mutants of *Drosophila melanogaster*, several sex-linked temperature-sensitive paralysis mutants have been isolated^{1,2}. These mutants are reversibly paralysed at 29° C while remaining normal at 22° C. The author set out to determine the molecular basis for the paralysis in one allelic series of these mutants, *shibire temperature-sensitive* (*shi*^{ts})².

A number of pharmacological agents are available which block particular functions of the nervous system. Here I report the effect of one such agent, tetrodotoxin (TTX), on the *shi* mutants. This toxin is known to specifically block the regenerative sodium channel of the action potential mechanism³.

Varying concentrations of TTX in 0.05 M citrate buffer pH 4.8 were fed to wild-type and *shi*^{ts} flies at 22° C. After 20 h, these flies were transferred to vials containing fresh food and 12 h later the number of live and dead flies was counted. In this way, kill curves were determined for the allele *shi*^{ts} and wild-type Oregon-R flies. The 12 h delay prior to scoring allowed those flies which were initially debilitated by the treatment to either recover or die. In most cases, debilitated flies recovered during this interval and so only two classes of fly were counted, dead or alive. The kill curves obtained for these two strains are shown in Fig. 1. The *shi*^{ts} flies are far more resistant to TTX than their wild-type counterparts, the LD₅₀ of *shi*^{ts} being three times greater than that of the wild-type strain.

To show that the result observed was due to the mutation at the *shi* locus and not just the genetic background of this strain, all of the other five *shi*^{ts} alleles² were tested for their resistance to the toxin. Table 1 shows the effect of feeding TTX at a concentration of 5 µg ml⁻¹ to flies carrying each of the *shi*^{ts} alleles and including a wild-type control. As might have been expected, the alleles defined as more extreme², *shi*^{ts1} and *shi*^{ts2}, conferred the greatest resistance while the least extreme alleles, *shi*^{ts3} and *shi*^{ts4}, exhibited very little resistance. Two unexpected results were that *shi*^{ts5}, an allele with an intermediate paralytic phenotype, was more resistant to TTX than *shi*^{ts1}, and *shi*^{ts6}, which is

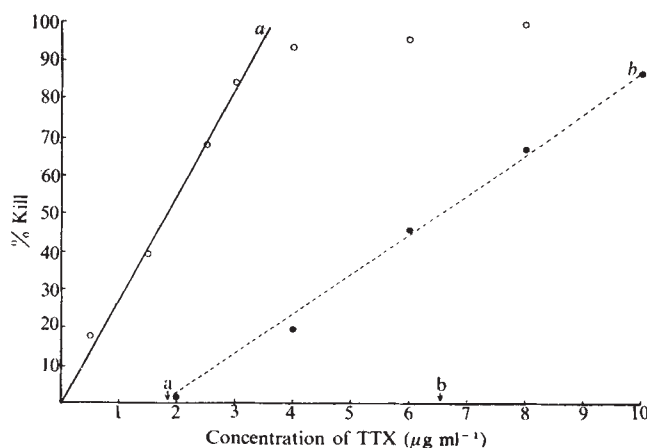


FIG. 1 Effect of increasing concentrations of TTX in 0.05 M citrate buffer pH 4.8, on *a*, Oregon-R, and *b*, *shi*^{ts1} flies at 22° C. Incubation time was 20 h. Each point represents ten vials, each vial containing fifty flies. For either *shi*^{ts1} or Oregon-R flies the variation in kill between vials at the same TTX concentration was always less than 5%. Arrows marked *a* and *b* indicate LD₅₀ for Oregon-R and *shi*^{ts1} flies respectively.

normally considered a strong allele, was the least resistant. Other studies on the paralysis and lethality of various heterozygous combinations of the *shi*^{ts} alleles have, however, shown that in *shi*^{ts5}/*shi*^{ts1} heterozygotes, the *shi*^{ts1} phenotype is expressed while in *shi*^{ts1}/*shi*^{ts1} flies, the *shi*^{ts1} phenotype is dominant (T. C. Kaufman and D. T. Suzuki, in preparation). In fact, *shi*^{ts5} and *shi*^{ts6} are recessive to all of the other *shi*^{ts} alleles. Furthermore, *shi*^{ts5} and *shi*^{ts6} are the only alleles which are viable in heterozygotes carrying a deficiency for the *shi* locus (T. C. Kaufman and D. T. Suzuki, in preparation). Thus, the sensitivity to TTX suggests a difference in properties of *shi*^{ts5} and *shi*^{ts6} which are corroborated by other criteria.

TABLE 1 Effect of TTX on *shi*^{ts} Alleles

Genotype	No. of flies		% kill
	Dead	Alive	
Oregon-R	300	11	96.5
<i>shi</i> ^{ts1}	188	284	39.8
<i>shi</i> ^{ts2}	156	344	31.2
<i>shi</i> ^{ts3}	210	285	42.5
<i>shi</i> ^{ts4}	308	184	62.6
<i>shi</i> ^{ts5}	298	190	61.1
<i>shi</i> ^{ts6}	392	103	79.1

5 µg ml⁻¹ TTX was used on mixed male/female populations. No preferential killing of males or females was observed.

Heterozygous *shi*^{ts1}/+ flies have also been tested for their TTX resistance. Table 2 shows the effect of 5 µg ml⁻¹ of TTX on these flies as compared to *shi*^{ts1}/*shi*^{ts1} and wild-type females. The TTX resistance of these heterozygous flies was found to be intermediate in value, being less than *shi*^{ts1} but greater than Oregon-R. This result correlates with the semi-dominant paralytic characteristics of *shi*^{ts1}, in that *shi*^{ts1}/+ heterozygotes are immobilised at 35° C but not at 29° C (L. E. Kelly, L. Hall, and D. T. Suzuki, in preparation).

It could be suggested that the resistance of *shi*^{ts} flies to TTX is simply a reflection of the difference in either their drinking behaviour or their ability to absorb the toxin through the gut wall. Consequently, the following experiment was performed. Bilateral mosaics were recovered from a cross of *shi*^{ts1} males to females carrying an unstable ring X chromosome (*In(1)w^{sc}*)⁴. Loss of this ring X chromosome shortly after

TABLE 2 Effect of 5 $\mu\text{g ml}^{-1}$ TTX on heterozygous *shi^{ts1}/+* females

Genotype	No. of flies		% kill
	Dead	Alive	
Oregon-R	14	212	93.8
<i>shi^{ts1}/shi^{ts1}</i>	176	63	26.3
<i>shi^{ts1}/+</i>	143	104	41.7

Controls were female. (The difference between the kill of *shi^{ts1}* females in this experiment and that shown in Fig. 1 is probably due to the use of a new batch of TTX for this experiment.)

fertilisation produces flies which contain cells which are heterozygous, *shi^{ts1}/+*, and hemizygous, *shi^{ts1}/0*, for the *shi^{ts1}* mutation. A mutation conferring yellow body colour was linked to the *shi^{ts1}*-bearing chromosome so that the genotype of the cells in the external chitin could be determined. Bilateral mosaics produced in this fashion have one side which is female and heterozygous for *shi^{ts1}* and the other side is yellow, male and genotypically *shi^{ts1}/0*. When shifted to 29° C, the *shi^{ts1}/0* half of the bilateral mosaic became paralysed, while the *shi^{ts1}/+* half remained normal. It has already been shown (Table 2) that *shi^{ts1}/+* flies are less resistant to TTX than hemizygous *shi^{ts1}/Y* flies. If, however, the resistance of *shi^{ts1}* flies to TTX was due solely to a reduction in the uptake of the drug, the haemolymph of the bilateral mosaic would be expected to contain a uniform distribution of TTX so that both halves of the animal would be equally affected. The bilateral mosaics were fed TTX at a concentration of 10 $\mu\text{g ml}^{-1}$ for 10 h. These conditions had previously been found to severely debilitate a population of *shi^{ts1}/+* heterozygotes without causing much death. In the mosaics so treated, the *shi^{ts1}/+* sides of the flies were debilitated as expected; however, the *shi^{ts1}/0* halves of the same animals remained unaffected. Thus, the debilitation of the mosaics after TTX feeding was the reverse of the paralytic effects observed when the mosaics were shifted to 29° C. This result shows that the difference in resistance between *shi^{ts1}* and wild-type to TTX is not due to a difference in the uptake of the toxin; rather, the *shi^{ts1}* tissue is indeed resistant to the molecule.

Although the neurophysiological effect of TTX is known, there is still the possibility that the TTX-induced lethality in *Drosophila* is not due to a malfunctioning of the nervous system, but to some secondary toxic effect of the drug. To eliminate this latter possibility, it is necessary to show some TTX resistance of *shi^{ts1}* flies in neurophysiological terms. This was done by comparing the effect of a controlled dose of TTX on the electroretinogram (ERG) of *shi^{ts1}* and Oregon-R flies. TTX eliminates the 'on' and 'off' transients of the d.c. recorded ERG of wild-type *Drosophila* (Fig. 2). The heads of ten *shi^{ts1}* and ten Oregon-R flies were each injected with 0.1 μl of a 25 $\mu\text{g ml}^{-1}$ solution of TTX in 0.6% NaCl and the ERGs were recorded. Within 5 min, nine out of the ten wild-type flies produced an ERG which did not possess the transients, while all ten of the *shi^{ts1}* flies retained the transients for longer than 30 min after the injection. While it cannot be said that each injected head retained all of the TTX, the result does indicate that those cells which give rise to these transients are more sensitive to TTX in Oregon-R flies as compared to the same cells in *shi^{ts1}* flies. It can be concluded from this experiment that the resistance to TTX which is conferred by the presence of the *shi^{ts1}* allele reflects an altered sensitivity of the nervous system to the toxin.

Since the *shi^{ts1}* alleles cause temperature-sensitive paralysis, it might be expected that the TTX resistance would also show a temperature dependence. As *shi^{ts1}* flies become paralysed when kept at temperatures in excess of 25° C for prolonged periods and as it has been found that 18° C rather

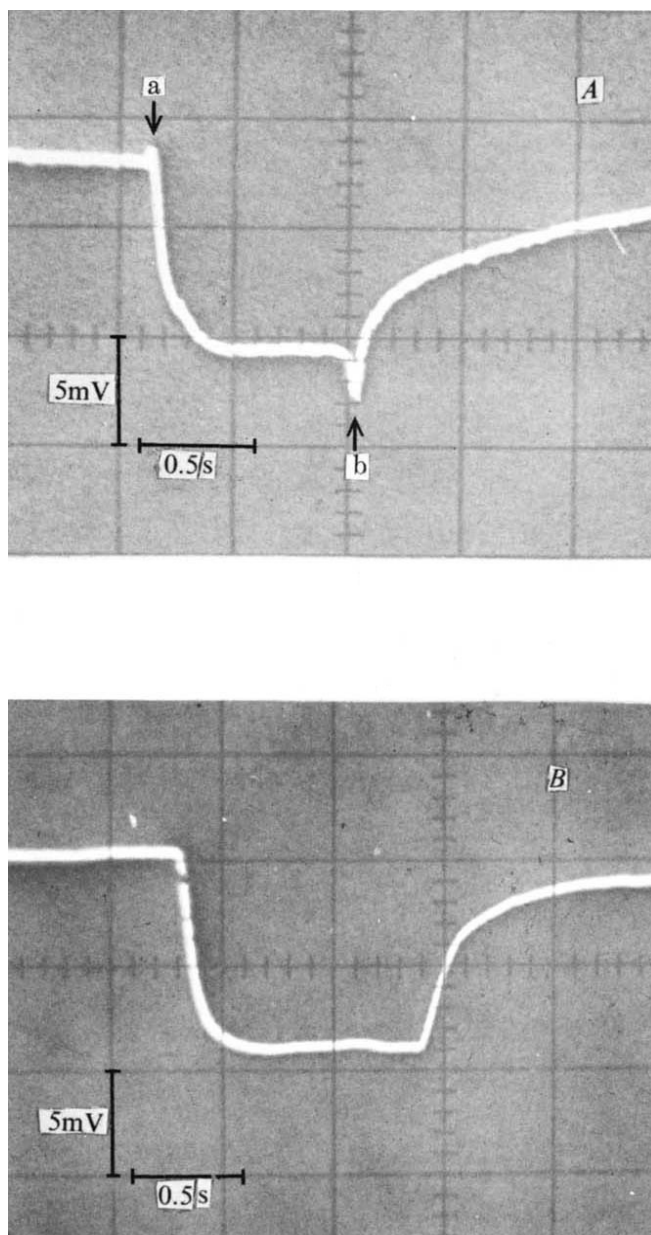


FIG. 2 Effect of TTX on the ERG of wild-type *Drosophila*. A, Normal ERG of a wild-type fly. Note the presence of the 'on' transient (a) and the 'off' transient (b). B, ERG from a wild-type fly injected with 0.01 μl of a 100 $\mu\text{g ml}^{-1}$ solution of TTX in 0.6% NaCl. Note the loss of the transients.

than 22° C is the true permissive temperature for *shi^{ts1}* (L. E. Kelly, L. Hall, and D. T. Suzuki, in preparation), the resistance of *shi^{ts1}* to TTX at 17° C and 22° C was compared. Kill curves were constructed for *shi^{ts1}* and Oregon-R at 17° C. As both strains seemed however to be more resistant to TTX at this temperature, the incubation time was extended to 48 h. This produced a kill curve for Oregon-R which is comparable to that obtained at 22° C. The curves obtained (Fig. 3) show that there is a large increase in the resistance of *shi^{ts1}* to TTX at this temperature, the LD_{50} of *shi^{ts1}* being some seven times greater than wild type. Preliminary investigations of *shi^{ts1}* flies at 22° C and 25° C indicate that *shi^{ts1}* is also more resistant at the lower temperature. Finally, if the *shi^{ts1}* flies are fed TTX at 22° C to the point of debilitation and then shifted down to 17° C, they recover from the debilitation

within 5 min, while no comparable effect is observed with Oregon-R flies. The converse is also true; *shi^{ts}* flies which are debilitated by TTX at 17° C are immediately paralysed and die when shifted to 22° C. This indicates two things; first, the debilitation induced by TTX is temperature reversible in *shi^{ts}* and secondly, the increased resistance of Oregon-R and, in part, of *shi^{ts}* at 17° C is probably due to a reduced rate of uptake of liquid in both strains. This may be a consequence of reduced dehydration at the lower temperature.

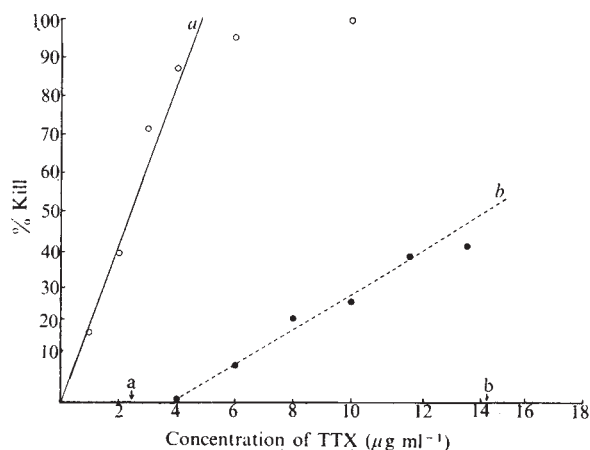


FIG. 3 Effect of increasing concentrations of TTX on *a*, Oregon-R, and *b*, *shi^{ts}* flies at 17° C. Incubation time was 48 h. Arrows marked a and b indicate LD₅₀ for Oregon-R and *shi^{ts}* flies respectively.

In conclusion, it has been shown that the *shi^{ts}* alleles confer on *Drosophila* a resistance to TTX which, like the paralysis of these flies, is temperature dependent. It has also been demonstrated that this resistance is attributable to an alteration in the properties of the nervous system. The TTX resistance results obtained for the *shi^{ts}* and *shi^{ts}* alleles are interpreted as resulting from alterations of different sites in the protein molecule encoded by this gene. It is suggested that the *shi^{ts}* locus is involved in the production of some component of the regenerative sodium channel and that the presence of the *shi^{ts}* allele, as well as producing temperature-sensitive paralysis, also reduces the affinity of this channel for TTX.

I thank Drs David T. Suzuki and Linda Hall for discussions. This research was supported by grants from the National Research Council of Canada and the National Cancer Institute of Canada to Dr Suzuki.

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Received October 18, 1973.

Dinosaur Monophyly and a New Class of Vertebrates

TRADITIONALLY dinosaurs are classified as two or three separate, independent groups of reptiles in the Subclass Archosauria. But evidence from bone histology, locomotor dynamics, and predator/prey ratios strongly suggest that dinosaurs were endotherms with high aerobic exercise metabolism, physiologically much more like birds and cursorial mammals than any living reptiles¹⁻⁸. Recently Ostrom has argued forcefully that birds are direct descendants of dinosaurs and inherited high exercise metabolism from dinosaurs^{1,8}. Here we present evidence that dinosaurs are a single, monophyletic group, and that the key advancements of endothermy and high exercise metabolism are justification for removing dinosaurs from the Reptilia and placing them with birds in a new class, the Dinosauria.

The two generally accepted orders of dinosaurs, the Saurischia and Ornithischia, are usually interpreted as independent derivatives of primitive thecodontian reptiles of the Triassic, but all known Triassic dinosaurs can be distinguished from typical thecodontians (Fig. 1)⁹⁻²³.

Most thecodontians had a wide-track sprawling gait or a crocodile-like semi-erect gait, both requiring much multidirectional mobility at the shoulder⁶. The thecodontian glenoid, or shoulder socket, was lizard or crocodile-like—being a saddle-shaped notch facing outward and backward, permitting humerus rotation, abduction, and backswing. Dinosaur glenoids were concave sockets facing mostly down and backwards, little outwards, restricting humerus movement severely to a fore-and-aft vertical plane—the 'fully erect gait'⁶.

Also, in thecodontians and reptiles, generally, the deltopectoral crest (*dp* in Fig. 1) is located close to the humerus head, giving the pectoralis musculature good leverage for rotating the humerus about its long axis but relatively little leverage for a vertical humerus backswing. In dinosaurs, the vertical backswing leverage was increased by moving the deltopectoral crest down the humerus shaft (Figs. 1 and 2).

In thecodontians and reptiles generally, the fourth trochanter (*4t* in Fig. 1) usually was located close to the femoral head, giving the caudifemoralis brevis musculature good long-axis rotational leverage, but little leverage for a vertical backswing. In dinosaurs, the apex of the fourth trochanter was developed into a distally directed flange, increasing the leverage for a vertical backswing (Figs 1 and 2).

A sprawling or semi-erect gait directs the thrust of the femur strongly inwards into the acetabulum, or hip socket. The thecodontian acetabulum usually was a strong, continuous bony surface as in most living reptiles. In dinosaurs, femoral action was more restricted to a vertical plane; the acetabulum was perforated to allow deeper penetration of the inturned femoral head, and the weight of the body was transmitted from the proximal surface of the femur to the strong dorsal rim of the acetabulum²⁴.

The thecodontian hand, known in phytosaurs²⁰, aetosaurs^{17,25}, rauisuchids²⁶ and 'Cheirotherium'^{27,28}, was crocodile-like with five long digits, the inner three subparallel, the outer two divergent (Fig. 1). The hand of Triassic saurischian dinosaurs is distinctive (Fig. 1) with a very short, stout metacarpal I; a long, strong thumb phalanx I; a powerful, curved, trenchant thumb claw; thumb articulations forcing the claw to diverge and point inwards during extension, converge with digits II and III and point downwards during flexion; long digits II and III, subequal and subparallel, bearing trenchant claws; and reduced digits IV and V²⁹. The hand of the better known primitive ornithischians (fabrosaurids and hypsilophodontids) differed from that of Triassic saurischians in lacking the long thumb and having blunt hooves instead of claws on digits I to III (ref. 30). Recently, a complete articulated skeleton of *Heterodontosaurus*, a Triassic ornithischian, was collected by A. W. Crompton, now at Harvard, for the South African Museum. The hand is virtually identical to that of

¹ Grigliatti, T. A., Williamson, R., and Suzuki, D. T., *Genetics*, **64**, S27 (1970).

² Grigliatti, T. A., Hall, L., Rosenbluth, R., and Suzuki, D. T., *Molec. gen. Genet.*, **120**, 107 (1973).

³ Narahashi, T., Moore, J., and Scott, W. R., *J. gen. Physiol.*, **47**, 965 (1964).

⁴ Hinton, C. W., *Genetics*, **40**, 951 (1955).

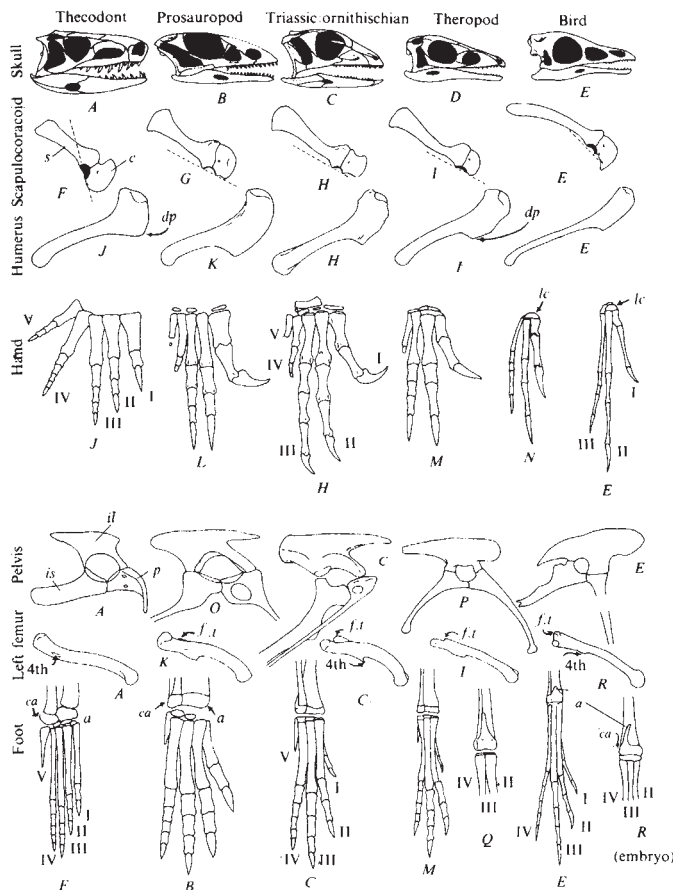


Fig. 1 Skulls, forelimbs and hindlimbs of thecodontians, dinosaurs and birds. All views of right elements except femur. (A) *Euparkeria*, (B) *Anchisaurus*, (C) *Fabrosaurus*, (D) *Compsognathus*, (E) *Archaeopteryx*, (F) *Gracilisuchus*, (G) *Plateosaurus*, (H) *Heterodontosaurus*, (I) *Halticosaurus*, (J) *Ticinosuchus*, (K) *Efraasia*³⁹, (L) *Thecodontosaurus*, (M) *Syntarsus*, (N) *Deinonychus*, (O) *Ammosaurus*, (P) *Coelophysus*, (Q) an ornithomimid and (R) *Grus*. *Anchisaurus*, *Fabrosaurus* (except skull), *Heterodontosaurus*, *Efraasia* and *Thecodontosaurus* from original material; data for the others from refs 20, 21, 26, 28, 36, 44, 53–55. (s) scapula, (c) coracoid, (dp) deltopectoral crest, (lc) lunate carpal, (il) ilium, (is) ischium, (p) pubis, (a) astragalus, (ca) calcaneum, (ft) femorotibialis, (4t) fourth trochanter, (---) long axis of glenoid.

Triassic saurischians in all six of the features cited above. Although specialised cranially³¹, *Heterodontosaurus* probably represents the original ornithischian hand pattern, inherited from saurischians, where the thumb was specialised for defence and digits II and III were used for defence, digging, and support during slow, quadrupedal locomotion. In more advanced ornithischian hands, the defensive function was lost, the thumb was secondarily shortened and simplified, and the claws were replaced by hooves, but in *Hypsilophodon*, digits II and III are still subequal and subparallel³⁰.

The origin of the femorotibialis, a knee extensor, in most thecodontians and in reptiles generally, is on the smooth anteriolateral surface of the femoral shaft. In primitive saurischians and ornithischians, the origin was expanded by the development of a spike-like ridge (*ft* in Fig. 1). In advanced saurischians and ornithischians, and in birds, this spike becomes a large crest—the 'lesser trochanter', not homologous to the lesser trochanter of mammals. Increased muscle power for knee extension probably was related to the development of the fully erect gait. Ornithosuchid thecodonts have this crest but differ from dinosaurs dramatically at the ankle and forelimb^{10,18}.

The ankle of most thecodontians was complex and crocodile-like: the astragalus articulated movably with the calcaneum and tibia, and the calcaneum bore a 'heel' (calcaneal tuber) for the gastrocnemius musculature^{24,32}. In advanced thecodontians the astragalus-calcaneal joint was a deep ball and socket³³. In sharp contrast, the dinosaur ankle was stiff, simple and bird-like: the astragalus and calcaneum were rounded caps fixed immovably on the ends of the tibia and fibula; the distal tarsals were immovable caps on the metatarsal heads, and the tarsal joint was a simple unidirectional hinge between astragalus-calcaneum and distant tarsals (Fig. 1)^{24,28}. Saurischian diphyly has been suggested because some theropods, such as *Allosaurus*, have deep ilia and a thin, anterior ascending process of the astragalus, while prosauropods have unexpanded ilia, no ascending process, and a peg-in-notch astragalus-tibial joint^{22,34}. Triassic forms, however, show how the transition from prosauropod-grade to advanced theropod grade saurischian occurred. The Triassic theropods *Syntarsus* and *Halticosaurus* had expanded ilia but retained a prosauropod-type astragalus-tibial joint without an anterior ascending process^{35,36}. In general, the prosauropod grade of postcranial anatomy seems to be the primitive dinosaur pattern. Details of tarsal structure are often obscured by incomplete ossification, but the pattern in *Syntarsus* is virtually identical to that of *Heterodontosaurus* (Fig. 1). Digit I arises two-thirds down the length of metatarsal II and is reduced and slightly divergent; digits II and IV are subequal; digit III is the longest; digit V is reduced to a splint; distal tarsal IV is cuboid; III is rectangular; II is a thin plate and the astragalus-calcaneum are prosauropod-like.

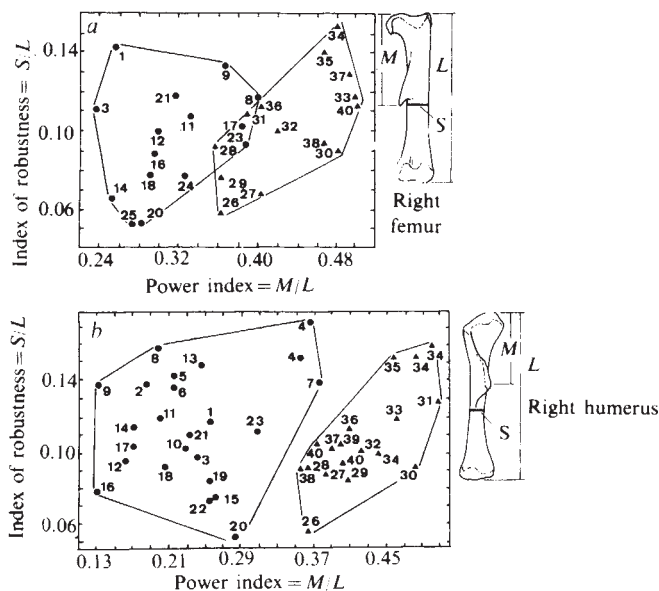


Fig. 2 Robustness and position of muscle attachments, (a) in the femur and, (b) in the humerus. The data are taken from specimens and refs 9, 10, 13, 15–20, 26, 28, 30, 36, 38, 39, 42, 43. Thecodonts, ●, are (1) *Proterosuchus*, (2) *Erythrosuchus*, (3) *Euparkeria*, (4) *Shansisuchus*, (5) *Fenhosuchus*, (6) *Wangisuchus*, (7) *Argentinosuchus*, (8) *Neoaeosauroides*, (9) *Stagonolepis*, (10) *Palaeorhinus*, (11) *Phytosaurus*, (12) *Rutiodon*, (13) *Myristosuchus*, (14) *Gracilisuchus*, (15) *Turfanosuchus*, (16) *Ornithosuchus*, (17) *Riojasuchus*, (18) *Ticinosuchus*, (19) *Sphenosuchus*, (20) *Pseudhesperosuchus*, (21) *Pedeticosaurus*, (22) *Triassolestes*, (23) *Alligator*, (24) *Lagosuchus*, and (25) *Lagerpeton*. Dinosaurs (▲), are (26) *Staurikosaurus*, (27) *Ischisaurus*, (28) *Herrerasaurus*, (29) *Halticosaurus*, (30) *Efraasia*, (31) *Massospondylus*, (32) *Teratosaurus*, (33) *Plateosaurus*, (34) *Riojasaurus*, (35) *Melanorosaurus*, (36) *Anchisaurus*, (37) *Fabrosaurus*, (38) *Heterodontosaurus*, (39) *Dysalotosaurus*, and (40) *Hypsilophodon*. (S) Minimum shaft diameter, (L) length of femur or humerus, (M) distance from head to muscle attachment (deltopectoral crest in humerus, fourth trochanter in femur).

The radiations of thecodontians and dinosaurs were based on different trends in locomotor anatomy, behaviour, and exercise metabolism. The earliest thecodontians, the proterosuchians¹⁵, had short limbs and a sprawling posture with a wide range of movement at each joint⁶. Stride length and power in the sprawling gait are increased by vertebral column undulations, and thecodontians generally lack adaptations for inhibiting lateral undulation. The complex ankle of advanced thecodontians was probably a response to selection for finer control of supination-pronation and greater leverage in sprawling locomotion. Thecodontian footprints²⁷ indicate that digit V in the hand and foot were strongly divergent and powerfully muscled, like digit I of climbing mammals. Flexibility at the major limb joints plus divergent digit V probably gave thecodontians a wide locomotor repertoire, including some digging, climbing, and movement over uneven ground. Some of the later Triassic thecodontians (such as aetosaurs and phytosaurs⁶) retained the sprawling gait and others, such as rauisuchids and ornithosuchids^{10,18}, developed a crocodile-like locomotor system which make possible both sprawling and semierect gaits^{6,37}. Inturned femoral heads indicate that a few thecodontians such as *Hallopus* and some advanced rauisuchids and ornithosuchids¹⁹, may have had narrow-tracked, fully erect hindlimbs, but even these forms retained the complex ankle.

The first well-known dinosaur is *Staurikosaurus*, of late Middle or early Late Triassic^{38,39}, a long-jawed predator with very long, gracile hindlimbs and postcranial anatomy of prosauropod grade (the femoral head is not as sharply inturned as in later dinosaurs and there is an oval acetabulum). More advanced theropods, such as *Ischisaurus* and *Herrerasaurus*, and ornithischians, such as *Pisanosaurus*, appear shortly after *Staurikosaurus*⁹. The original dinosaur stock were probably predators with higher running speeds and exercise metabolism than in thecodontians of comparable size. Early dinosaurs may have been bipedal at top speed, but the strong forelimb modifications for the fully erect gait and the prosauropod-like hand indicate that quadrupedal progression was very important. Even at the prosauropod grade, dinosaur bone histology was identical to that of endothermic mammals⁴. Restriction of joint movement to a fore and aft, vertical plane and the stiff, bird-like ankle would have made climbing difficult for any dinosaur⁴⁰. The vertebral column of early dinosaurs was stiffened to inhibit lateral undulations by the development of extra vertebral articulations, the hyposphene-hypantra in saurischians, and long ossified tendons appressed against the neural spines in ornithischians. The locomotor repertoire of dinosaurs was narrow, rather restricted to moving over fairly even terrain. Recent experiments⁴¹ show that erect locomotion does not require less energy for a given speed than sprawling, as has usually been assumed⁶, but erect locomotion probably does increase manoeuvrability at very high speeds. The dinosaur radiation was based on a concentration of behavioural, physiological, and anatomical adaptations for high, sustained running speeds which made them irresistible predators and competitors to contemporary thecodontians and the larger mammal-like reptiles.

Could the similar postcranial adaptations of Triassic dinosaurs reflect merely convergent evolution of the erect gait from different thecodontians? We believe that the answer to this question is almost certainly no. Evolution of erect posture does not invariably lead to an avian-dinosaur type joint pattern. Some thecodonts and many mammals, such as carnivores and ungulates, have a fully erect gait but retain complex ankles with astragalar-calcaneal mobility and calcaneal 'heels'. The very detailed similarity in all the major joints, especially the hands and feet, of Triassic ornithischians and saurischians makes dinosaur polyphyly exceedingly unlikely. Moreover, the pattern of increasing dinosaur diversity in the Triassic upwards from the stratigraphic level of *Staurikosaurus* suggests one monophyletic radiation. Some of the little 'rabbit thecodontians' such as *Lagosuchus* and *Lagerpeton*^{42,43}, have im-

mobile astragalar-calcaneal joints and lack calcaneal 'heels', and may be related in some way to dinosaur origins.

The prosauropod saurischians have a mixture of primitive and advanced features which show how ornithischians evolved from saurischians (Fig. 1). In the primitive ornithischian dentition, represented by *Fabrosaurus*, the anterior teeth were non-serrated, pointed and slightly incurved with swollen bases and the cheek teeth were serrated with triangular crowns⁴⁴. In some prosauropods, such as *Massospondylus* and probably *Anchisaurus*, the basic pattern was similar: anterior, simple teeth and triangular, serrated cheek teeth. In advanced prosauropods, such as *Plateosaurus* and in most ornithischians, the jaw articulation was depressed below the tooth row level, but in *Fabrosaurus* and *Anchisaurus* the articulation was still on the same level as the tooth row⁴⁴. The lower jaw of most ornithischians was deep and massive, but the fabrosaur jaw was slender like that of *Anchisaurus*. In early ornithischians the origin of iliotibialis, a knee extensor co-inserting with the femorotibialis, was expanded by the development of a long anterior iliac prong. Such a prong is unknown in thecodontians and most prosauropods, but is present in very fabrosaur-like configuration in *Anchisaurus* and *Ammosaurus*⁴⁵. In ornithischians the primitively broad, plate-like ventral surface of the pubis and ischium were deeply excavated and reduced; such excavations had begun in the prosauropods *Anchisaurus* and *Ammosaurus*⁴⁵.

The prosauropod grade is quite primitive, and herbivorous prosauropods must have diverged very early from the basal, predatory dinosaur stock. It should be noted that no thecodontians show trends away from carnivory towards herbivory, except the aetosaurs, sprawling, armoured types totally unlike ornithischians¹⁷. Small prosauropods with *Anchisaurus*-type jaw articulations, ilia and pubes were probably the immediate ancestors of ornithischians. The adaptive shift probably was an emphasis on ultra-high speed bipedality plus adaptations for prehension and cutting of plant fibres. Excellent fabrosaur material collected for the South African Museum and now at Harvard, shows that the development of an upper beak had just barely begun in *Fabrosaurus*. The fabrosaur premaxillary was small, sharply pointed, and movably articulated with the dentary; the dentary symphysis was mobile. The premaxillary beak could serve as a gouge for digging into plant stems and as a cutting edge occluding with the arcade of premaxillary teeth, much like the incisors of kangaroos⁴⁶, while the mobile symphysis permitted the retention of independent control of each jaw ramus, important for tooth-to-tooth shearing on one side of the mouth at a time. Excavations in the maxilla and dentary, for cheek pouches to retain the cut ends of plant material⁴⁷, had begun in *Fabrosaurus*. All known Triassic ornithischians had very long hind limbs with elongate distal extremities indicating very high speeds. A short trunk facilitates the maintenance of balance in a highly bipedal animal, but herbivory demands a long, bulky gut. Ornithischians solved the balance problem by rotating the pubis posteriorly so that the distal end, marking the end of the gut, lay far posterior to the acetabulum. Thus, the gut was extended between the hindlimbs and the pre-acetabular trunk section could be abbreviated⁴⁸.

Although Walker⁴⁹ has cited some resemblances between living birds and crocodile-like thecodontians (sphenosuchids), we believe that Ostrom has shown that the similarities between *Archaeopteryx* and small theropods are so detailed and comprehensive that the immediate ancestor of birds must have been a saurischian dinosaur^{1,8,50}. Some of the features cited by Walker as evidence of crocodile-bird relationships are actually widespread among tetrapods: cranial salt glands are developed in lizards, especially in *Amblyrhynchus*⁵¹; forearm articulations which force the radius distally during forearm flexion are present in salamanders, *Sphenodon*, and lizards. Although details are obscure, the *Archaeopteryx* skull seems much more like the light, kinetic skulls of small theropods than the solidly braced sphenosuchid structure⁵². *Archaeopteryx*

has the complete suite of seven features listed above as diagnostic of the primitive dinosaur erect gait. In flying birds, bats and pterosaurs the glenoid faces strongly outwards to make possible the abduction of the humerus necessary for gliding and powered flight. Significantly, *Archaeopteryx* retained the typical, downward-facing dinosaur glenoid; it is difficult to imagine how this joint would have made either flight or gliding possible. The initial evolution of feathers probably was not associated with flight, a hypothesis developed at length by Ostrom¹.

Ostrom has shown that the hands and feet of *Archaeopteryx* are identical in detail to certain Jurassic/Cretaceous theropods, and differ only in being larger relative to the body size (Fig. 3)^{1,50,53-55}. Feathers may have been widespread in bird-like theropods. *Archaeopteryx* strongly resembles small theropods in general body plan with a short trunk, thin, flexible neck, very long hindlimbs and long fingers bearing trenchant claws for snagging prey. Sphenosuchid thecodontians were quite different (Fig. 3) with a long trunk, thick neck and long forelimbs with short digits adapted for running and not prehension.

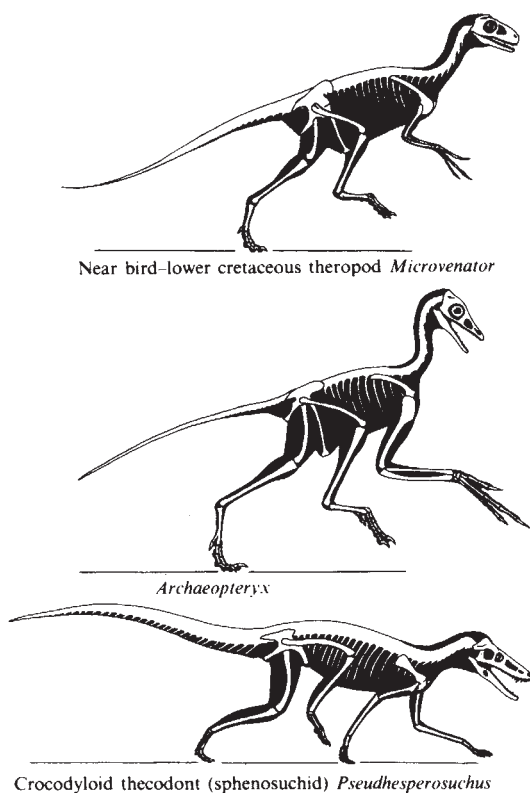


Fig. 3 Body form in a small theropod dinosaur, the first known bird and a crocodyloid thecodontian. *Microvenator* hand and skull restored after *Ornitholestes*. Data from refs 10, 53-55.

Endothermy and high aerobic exercise metabolism are sufficient justification for separating birds into a class distinct from other living sauropsid tetrapods. But endothermy and high exercise metabolism were probably already present in the dinosaur ancestors of birds and are the key features differentiating dinosaurs from crocodilians and the other extinct archosaurs. The bird radiation has produced many species, but the structural diversity is not more striking than that within the Ornithischia or Saurischia. Bird physiology is also rather stereotyped. The avian radiation is an aerial exploitation of basic dinosaur physiology and structure, much as the bat radiation is an aerial exploitation of basic, primitive mammal

physiology. Bats are not separated into an independent class merely because they fly. We believe that neither flight nor the species diversity of birds merits separation from dinosaurs on a class level. Among all amniotes, the most profound adaptive shift was from ectothermy to endothermy, which occurred during the origin of mammals and dinosaurs. Therefore we propose the erection of a Class Dinosauria, to include as subclasses the Saurischia, Aves and Ornithischia. The currently recognised suborders of dinosaurs would be elevated to orders (Fig. 4). Thecodontians, crocodilians, and pterosaurs

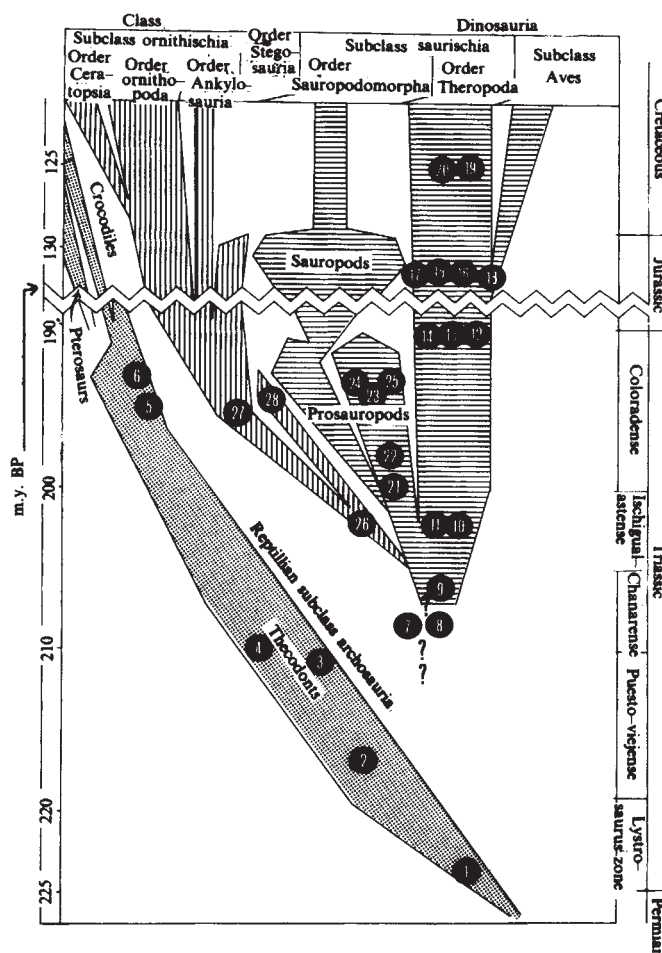


Fig. 4 Suggested phylogeny and classification of dinosaurs. Position of *Lagosuchus* and *Lagerpeton* is very uncertain. Within orders, ancestor-descendent relationship is not necessarily indicated by stratigraphic level of known specimens, for example, *Pisanosaurus* is earlier than, but in some ways more advanced than the earliest known *Fabrosaurus*. (1) *Proterosuchus*, (2) *Euparkeria*, (3) *Ticinosuchus*, (4) *Gracilisuchus*, (5) *Sphenosuchus*, (6) *Pseudhesperosuchus*, (7) *Lagerpeton*, (8) *Lagosuchus*, (9) *Staurikosaurus*, (10) *Ischisaurus*, (11) *Herrerasaurus*, (12) *Coelophysis*, (13) *Syntarsus*, (14) *Halticosaurus*, (15) *Coelurus*, (16) *Ornitholestes*, (17) *Compsognathus*, (18) *Archaeopteryx*, (19) *Microvenator*, (20) *Deinonychus*, (21) *Thecodontosaurus*, (22) *Efraasia*, (23) *Plateosaurus*, (24) *Ammosaurus*, (25) *Anchisaurus*, (26) *Pisanosaurus*, (27) *Fabrosaurus* and (28) *Heterodontosaurus*.

would remain in the reptilian subclass Archosauria, which would stand to Dinosauria much as the reptilian subclass Synapsida (the mammal-like reptiles) stands to Mammalia. This new classification, we believe, reflects more faithfully the major evolutionary steps. Ectotherms and forms transitional to endotherms are retained in the Reptilia and the two highly successful endothermic groups, mammals and dinosaurs, are given separate class status.

We thank J. Ostrom for data on *Archaeopteryx* and A. W. Crompton for access to fabrosaurid and heterodontosaurid material. Supported in part by the National Science Foundation.

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Received June 25; revised October 8, 1973.

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Viroids and viral hepatitis in marmosets

DIENER¹ and Zuckerman² have recently speculated that hepatitis A and B, respectively, may be caused by infective naked nucleic acids or viroids. In response to their hypotheses, we felt it would be of interest to investigate the nature of an agent known to cause hepatitis in marmoset monkeys. We reasoned that if the aetiological agent of hepatitis found in serum is a free, or naked, nucleic acid in this system, then its infectivity should be destroyed by the action of a specific endonuclease. For example, a free viral DNA would be rendered uninfected after treatment with pancreatic DNase. The virus used in our study was the 'Barker' agent, originally recovered by Deinhardt *et al.* from acute phase sera of marmosets inoculated with serum from a human case of viral hepatitis³. This virus consistently induces hepatitis in such animals, although its pedigree as a human hepatitis agent has been a subject of controversy⁴.

As many animal sera contain RNase activity, but not DNase activity, we assumed the postulated viroid for Barker-induced marmoset hepatitis, if it existed, would most likely be a naked DNA. To test this assumption, we divided a pool of infective acute-phase marmoset serum into two equal aliquots. One aliquot was treated with pancreatic DNase (EC 3.1.4.5) at 20 μ g ml⁻¹ for 1 h at 37° C; the other aliquot was heated to 37° C for 1 h without any other treatment. Three *S. nigricollis* marmosets were inoculated intravenously (i.v.) with treated serum (0.25 ml each); three other *S. nigricollis* marmosets were inoculated i.v. with untreated serum. The course of infection in each marmoset was monitored by measuring serum glutamic pyruvic transaminase (SGPT) and serum isocitric dehydrogenase (SICD) activities as indicator enzymes for liver damage.

All six marmosets demonstrated elevated SGPT and SICD activities by the fourth or fifth week after inoculation, indicating liver damages had been sustained in each animal. Clearly, then, the marmoset hepatitis agent cannot be a DNA viroid as all three animals receiving DNase treated serum showed convincing enzymatic evidence of viral hepatitis. The results of our preliminary study are consistent with the tentative finding by Deinhardt *et al.*⁵ that the Barker hepatitis agent bands in CsCl at a buoyant density of 1.2. Viruses usually band around $\rho = 1.2-1.4$, whereas free viral DNA bands at about $\rho = 1.7$.

While the possibility still exists that the marmoset hepatitis agent is another type of viroid, that is, a unique double-stranded RNA (or RNA:DNA hybrid)-protein conjugate which bands in CsCl at $\rho = 1.2$, our experimental evidence at least rules out the presence of a simple DNA viroid in serum containing the marmoset agent.

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Nutrient transfer between the seagrass *Zostera marina* and its epiphytes

THE leaves of seagrasses often harbour dense populations of macroscopic and microscopic algal epiphytes. In many cases seagrasses grow in waters that are extremely low in dissolved inorganic nutrients yet they have high epiphyte standing stocks. These field observations led to direct experiments on the transfer of nitrogen and carbon from the dissolved nutrient pool in the interstitial waters of the sediments into the root system of eelgrass, *Zostera marina*, and through the plant to the algae on its leaves. Previous studies^{1,2} on seagrasses have shown that the high productivity of seagrass meadows is largely maintained by the nutrient pool of the sediments. Since the plant system leaks phosphorus and, presumably, other nutrients, it is possible that the production of the leaf epiphytes is indirectly sustained by the nutrients in the sediments. This mechanism has been suggested by several workers for marine and freshwater macrophytes³⁻⁷ but definitive experiments are lacking. Here we report the results of experiments designed to test this hypothesis.

Eelgrass, *Zostera marina* L., was collected from beds on Visovodof Island in the Aleutian Islands of Alaska, on August 6, 1972, a few hours before experiments began. Sediments were removed from the roots, and then plants were placed in partitioned plexiglass containers in which the water surrounding the leaves was isolated from that surrounding the roots and rhizomes (Fig. 1). Each experimental container held three plants with their natural epiphyte populations. Containers were filled with 3 l of filtered seawater in the upper level and 1.5 l in the lower. Since the plants grow in anoxic sediments, the lower water surrounding the roots and rhizomes was stripped of dissolved oxygen by bubbling with a helium-carbon dioxide gas mixture before the experiment. The seal round the plant between water levels was maintained by a septum stopper fitted to the lower stem and sealed by silicone stopcock grease.

We added $^{15}\text{N-NH}_4^+$, $^{15}\text{N-NO}_3^-$, or $^{15}\text{N-(NH}_2)_2\text{CO}$ to the lower compartment of each container in low (33 $\mu\text{g atom N l}^{-1}$) or high (67 $\mu\text{g atom N l}^{-1}$) concentration. In addition, 20 μCi of ^{14}C -labelled HCO_3^- was also added to the lower compartment of each container. The experimental containers were incubated for 8 h under natural light and ambient temperatures (about 12° C). The lower compartment of all containers was darkened.

At the end of incubation the plants were removed from a container, one was bisected into leaves and roots-rhizomes and frozen immediately, and the other two plants were dissected into leaves, stem, and roots-rhizomes and dried at 60° C for 24 h. The epiphyte community of a leaf was

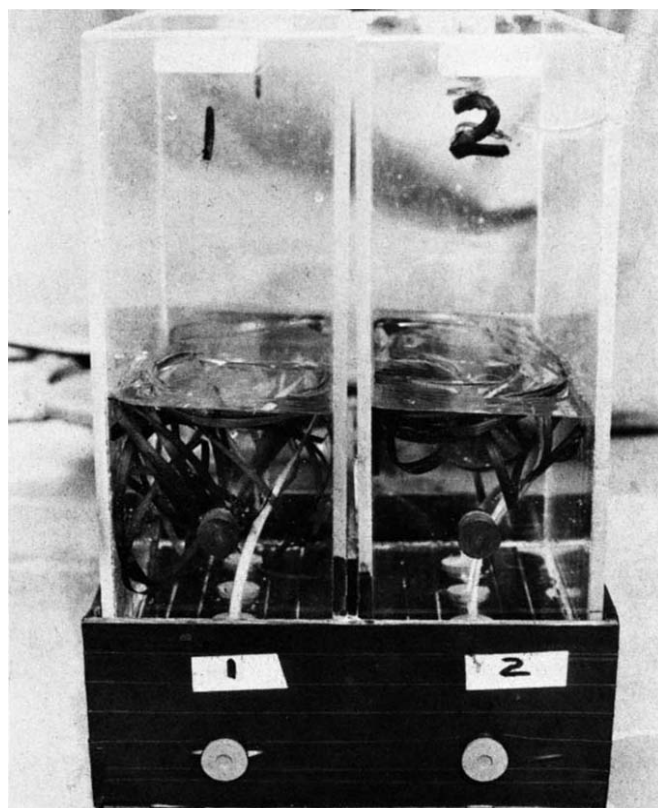


FIG. 1 Partitioned container with *Zostera* for measuring uptake of carbon and nitrogen. Roots and rhizomes are in the lower blackened compartment separated at leaf base by septum stoppers; only the portion of the plant above the septum stopper is visible.

sampled by wiping the leaf on a glass slide and washing the algae removed on to a silver filter. After filtering, the epiphyte samples were dried at 60° C for 24 h and retained in a desiccator until analysed first for ^{14}C and then for ^{15}N content. The epiphytes comprised mainly several species of diatoms.

Carbon uptake by *Zostera* was measured by converting organic carbon to CO_2 by combustion and the radioactivity of the $^{14}\text{CO}_2$ determined in a liquid scintillation counter after the method suggested by Wetzel⁸ for aquatic plants. The ^{14}C content of the epiphytes was determined by Geiger-Mueller counting in a procedure similar to that for phytoplankton productivity⁹.

Nitrogen uptake was measured by determining the quantity of ^{15}N in the plant and epiphyte material. The organic

TABLE 1 Carbon and nitrogen uptake by roots of *Zostera marina* L. and transport through the plant to leaf epiphytes. Carbon values are single observations; nitrogen values are means of two observations.

	Carbon uptake ($\mu\text{g g}^{-1} \text{h}^{-1}$)				Nitrogen uptake (10^2 % uptake h^{-1})			
	Roots and rhizomes	Stems	Leaves	Leaf epiphytes*	Roots and rhizomes	Stem	Leaves	Leaf epiphytes
Experiment I ^{15}N added as NH_4^+								
Low [N]	2.69	1.76	0.07	(0.03)	3.35	0.39	0.32	7.8
High [N]	11.83	0.27	0.13	(0.01)	4.16	1.01	0.25	7.2
Experiment II ^{15}N added as NO_3^-								
Low [N]	1.23	1.58	0.17	(0.01)	1.32	1.32	1.50	21.9
High [N]	5.91	0.24	0.08	(0.04)	1.82	1.33	1.88	6.5
Experiment III ^{15}N added as $(\text{NH}_2)_2\text{CO}$								
Low [N]	—NO DATA TAKEN—				0.025	0.033	0.051	8.7
High [N]					0.031	0.038	0.048	7.3

* Units for carbon uptake by epiphytes are μg per g leaf per hour.

Low [N] = 33 $\mu\text{g atoms N l}^{-1}$.

High [N] = 67 $\mu\text{g atoms N l}^{-1}$.

nitrogen was converted to N_2 by an automated Dumas technique¹⁰ and the ^{15}N content of the N_2 subsequently determined in a Bendix mass spectrometer. Uptake calculations followed the procedure of Dugdale and Goering¹¹. The data are presented as % uptake, and the relative velocity of nitrogen uptake; absolute uptake is obtained by multiplying the latter by the nitrogen content of the material.

The results of all experiments indicate a direct transfer of carbon and nitrogen from *Zostera* to the epiphytes on its leaves. ^{14}C labelled HCO_3^- and ^{15}N labelled NO_3^- , NH_4^+ , and $(NH_2)_2CO$ were all absorbed from solution by the root-rhizome system of the plant and the labels were subsequently transported to all parts of the plant and to the epiphytes on the leaves (Table 1). In all experiments after 8 h the ^{15}N content was larger in the epiphytes than in any part of the plant. Within a plant, a ^{15}N concentration gradient was observed with greatest amounts in the root-rhizome system and least in the leaves. The distribution of ^{14}C was generally similar to that of ^{15}N .

These results are positive evidence for the transfer of nitrogen from the sediments to the epiphyte community of a rooted macrophyte and could well be part of the explanation for how high standing stocks of epiphytic algae are maintained in waters with low concentrations of dissolved inorganic nutrients. It is possible that nitrogen and carbon are transferred directly from *Zostera* to the epiphytes as organic compounds, but transfer probably occurs by leakage of organic or inorganic compounds from the plant and subsequent uptake by the algae.

Previous experiments have shown that phosphorus absorbed by the root-rhizome system was in part lost across the leaves, so it is likely that this nutrient is also available for epiphyte growth. Harlin⁴ has shown that ^{14}C and $^{32}P-PO_4$ could be transferred from *Zostera* to the algal epiphyte *Smithora naiadum*. In addition, Brylinsky³ has shown that several species of tropical seagrasses lose about 2% of the carbon fixed in photosynthesis across the leaves; the loss was in the form of low molecular weight organic compounds. The excretion of organic carbon and nitrogen has been measured in rooted freshwater macrophytes⁵⁻⁷.

It is likely that *Zostera* and most other seagrasses obtain their nutrients from the sediments through the roots. There is also evidence that some inorganic carbon is taken up by the roots and transported to a site where it can be fixed in photosynthesis. This mechanism has been found in some species of vascular plants in freshwaters¹².

Our work indicates a symbiotic relationship between seagrasses and their epiphyte community. This implies complexities in nutrient and productivity cycles in shallow waters dominated by rooted vascular macrophytes. Clearly, epiphyte productivity is enmeshed with that of its associated macrophyte.

This study was supported by a grant from the Oceanography Section, National Science Foundation.

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Received August 20; revised November 26, 1973.

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Why kangaroos hop

KANGAROOS (family Macropodidae, order Marsupialia) are the only known group of large Australian herbivorous marsupials ever to have evolved major cursorial specialisations. During fast locomotion macropodids progress by a ricochet (bipedal saltation) involving a series of rebounds in which the two hind feet strike the ground at the same moment or practically synchronously and the forefeet not at all¹. In macropodids the axis of the hind foot is through digit IV which is unique among large cursorial mammals; perissodactyls and most rodents have the axis through digit III, while in artiodactyls, carnivores and leporids it lies between digit III and IV². These differences are the result of preadaptive complexes in the immediate ancestors of these groups.

The earliest placentals were probably terrestrial³ and the pes was primitively pentadactylous with a nonserial tarsus^{4,5}. Two basic vectors of weight transfer from the tibia to the digits were involved (Fig. 1A), one through the astragalus-navicular-cuneiforms-digits I-III, and the other through the astragalus-calcaneum-cuboid-digits IV-V. The non-serial tarsus made possible the option of a third vector of weight transfer which could pass through the astragalus to the cuboid and digits IV-V. The primitive tarsal configuration made possible a more or less equal distribution of weight transfer to each of the digits. Artiodactyls exploited the non-serial tarsus in development of a paraxonic foot with digits III-IV becoming the main supporting digits (Fig. 1B). In the development of a mesaxonic foot perissodactyls have reduced the astragalo-cuboid contact such that the tarsus is functionally serial⁵ (Fig. 1C). Cursorial specialisation in large placental herbivores are thus built upon the primitive placental foot structure. Placental carnivores have essentially retained the primitive tarsal arrangement with modifications being most evident in the reduction of lateral digits.

The first marsupials were probably arboreal⁶⁻⁸ and most likely had a hind foot structure similar to the living South American didelphid *Marmosa*⁹. The primary structure of the marsupial pes is adapted toward prehension, and its further specialisations reflect this derivation. Primitively the marsupial pes had a moderately large opposable hallux and digits II-V were of subequal size and length. The primitive marsupial tarsal arrangement has not yet been established although a non-serial tarsus seems most probable. The vector of weight transfer from the tibia to the digits was probably similar to that of primitive placentals.

Two major groups of pedis occur in Australian marsupials¹⁰. In the family Dasyuridae (marsupial carnivores) there is an elongation of the pes and parallel arrangement of the digits as well as recession of the hallux (Fig. 1D). The ancestral forms of this family seem to have abandoned prehensile modifications for semi-cursorial ones, the pes having been made servicable for rapid progression either in the trees or on the ground. Their terrestrial ancestors have substituted digitigrade for plantigrade progression⁹. Dasyurids typically have digits II-V of subequal size although there is a tendency for digits III-IV to be slightly larger. The vectors of weight transfer are thus similar to

those in placental carnivores. In addition, the primitive dasyurid pes has a pre-adaptive potential for evolution of major cursorial specialisations similar to those seen in placental ungulates; a potential which, however, has never been exploited.

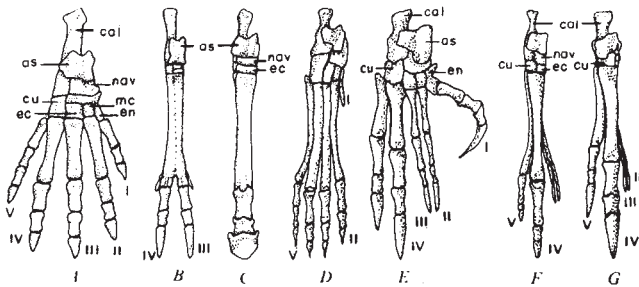


Fig. 1 Mammalian pes (right). A, Hypothetical placental ancestor; B, artiodactyl; C, perissodactyl (*Equus*); D, dasyurid (*Dasyurus*); E, phalangerid (*Trichosurus*); F, peramelid (*Macrotis*); G, macropodid (*Macropus*). A, B, C, F and G after ref. 15. Abbreviations: as, astragalus; cal, calcaneum; cu, cuboid; ec, ectocuneiform; en, entocuneiform; me, mesocuneiform; nav, navicular; digits I, II, III, IV, V.

The second line of Australian marsupial hind foot specialisation occurs in bandicoots (family Peramelidae), diprotodonts (family Phalangeridae and its derivatives) and in the common ancestor of these two groups. Digits II-III are reduced in size and bound together in the whole of their length by a skin sheath with the exception of their end joints and claws. This syndactylous condition is deemed to be a further arboreal adaptation^{7,8} and probably arose only once in the Australian marsupials⁹⁻¹⁵, although a diphyletic origin has been advocated^{16,17}.

In phalangerids (Fig. 1E), their immediate ancestors and the immediate ancestors of peramelids, digit IV is the major support digit of the hind foot, followed by digit V, then the hallux; digits II-III are very small and have ceased to play any part in walking, running or climbing and thus do not share in the more obvious functions that are associated with toes. The syndactylous digits have become a secondarily specialised toilet implement¹³. The major vector of weight transfer is through the astragalus-calcaneum-cuboid-digit IV, although part of the body weight is also carried through the astragalus-navicular-digits I-III vector. The common phalangerid-peramelid stock had a polyprotodont dentition¹¹.

Shortly after the acquisition of syndactyly, peramelids split off as a separate lineage from the syndactylous stock¹⁰. Peramelids retained a polyprotodont dentition⁹. Some peramelids progress by quadrupedal saltation (the spring) (for example, *Isodon*) while others (for example, *Choeropus*) have become functionally monodactylous quadrupeds (Fig. 1F). In all peramelids digit IV is the main support digit of the hind foot, a character inherited from their arboreal, syndactylous, ancestors. Peramelids have deviated from the phalangerid tarsal configuration in rearrangement of the cuboid, ectocuneiform and metatarsal IV such that the ectocuneiform has been incorporated into partial support of metatarsal IV. The tarsal configuration in peramelids allows the greater part of the body weight of the animal to pass directly from the astragalus to the distal tarsals bypassing to a great extent the calcaneum¹⁵. As a consequence the syndactylous pes of peramelids is, in part, functionally convergent with ungulates. These particular tarsal modifications, found only in the Peramelidae, have allowed a small syndactylous marsupial to become a rather specialised cursorial quadruped. This tarsal configuration would, however, be impractical for body weight transmission in a large animal. Such changes in the peramelid tarsal configuration have

been influenced by the antecedent development of syndactyly¹⁸. Peramelids and macropodids represent two independent terrestrial adaptive radiations from arboreal ancestors; similarities in foot structure are thus the result of convergence⁹.

Macropodids evolved from phalangerids after the acquisition of a diprotodont dentition⁹. The potential for cursorial specialisations in macropodids was set by the preadaptive plasticity of the phalangerid foot structure for terrestrial locomotion. As digit IV was the major supporting digit in the phalangerid pes, cursorial specialisations would have to be built around this digit in macropodids (Fig. 1G). Evolution of weight transfer vectors in macropodids incorporating digit III was restricted by the presence of syndactyly of digits II-III in their phalangerid ancestors. This restriction excluded the possibility of evolution of efficient quadrupedal cursorial specialisations similar to those occurring in placental ungulates where digit III is partly (artiodactyls) or wholly (perissodactyls) incorporated as a major support digit. Utilisation of digits III-IV together in a support function could not have evolved in macropodids¹⁴. In ungulates a vector of weight transfer is incorporated which passes weight from the astragalus to the anterior tarsals and bypasses the calcaneum⁵. Macropodids have, however, exploited the vector through the astragalus-calcaneum-cuboid-digit IV and deviated from the phalangerid tarsal arrangement in the relationship between the astragalus, navicular, cuboid, and calcaneum; in macropodids the astragalo-calcaneum and cuboid-calcaneum contact are greatly enlarged while the astragalo-navicular contact is reduced. In macropodids there is no ectocuneiform-metatarsal IV contact. This is in contrast to peramelids. Incorporation of the calcaneum into the primary weight transfer vector is a common feature among ricochet mammals^{19,20}, while in digitigrade and unguligrade mammals there has been a progressive evolution to exclude this bone from the role of supporting body weight²¹. The macropodid tarsal structure allows effective ricochet function by transfer of weight through the calcaneum. The fact that their phalangerid ancestors had already developed syndactyly and the use of digit IV as their main support digit thus predetermined the type of cursorial specialisation possible in the macropodid hind limb.

These observations provide an explanation for Howell's statement²² that "In the case of the . . . kangaroo, it is doubtful whether it is sufficiently effective at locomotion to justify the evolutionary effort put into it . . . A kangaroo is an amazingly specialised animal, but its method of traveling by saltation was hardly begun for the purpose of ultimate speed. Rather has it built speed into the locomotory pattern that was already established, probably for some other purpose".

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Received October 24; revised November 26, 1973.

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Banded mongoose males guard young

In most mammals care of the young is the prerogative of the female. The male, if present at all, contributes little. In the banded mongoose (*Mungos mungo*), however, males play an important role in guarding young at the den while the lactating females forage.

The banded mongoose is a diurnal social carnivore occurring in savanna habitats throughout much of Africa south of the Sahara. In Rwenzori National Park, Uganda, where I studied it for 2 yr, group (pack) size varied from approximately 5 to 40 animals. Reproduction within a pack was synchronised; several females typically came into oestrus at about the same time and subsequently produced their litters within a few days of each other. The young suckled indiscriminately from any lactating female and were groomed, played with, and transported by all pack members. They first began to leave the den for short afternoon foraging expeditions when 3 to 4 weeks old and at 5 weeks began to regularly accompany the adults in the morning. Until this age one or more pack members typically stayed at the den with the young when the others left for the morning's foraging. The pack usually returned after 2 to 4 h and for the remainder of the day the mongooses foraged intermittently in small groups. Occasionally however, a pack remained away from the den for an entire day.

To determine the sexes and identities of mongooses stay-

data for the 32 d in which marked animals stayed with the young. Twenty individuals were recorded guarding a total of 48 times. Adult males stayed with the young most frequently accounting for 73% of the total guarding records; 85% of the 20 mongooses which stayed alone with the young were adult males. The 9 marked lactating females were never recorded to stay with the young in the morning. Non-lactating females and animals less than 1 yr old occasionally guarded, but the latter did not remain alone with the young. Although the sample is small, it indicates that certain individuals show a greater tendency to stay with the young than others. In the E pack a male and non-lactating female accounted for 12 of the 17 guarding observations and two males accounted for 9 of the 11 observations in the F pack. Guarding was more equitably distributed in the I pack and 11 of the 17 pack members stayed with the young on at least one occasion.

The adaptive significance of guarding in the banded mongoose seems clear. Survival of young in the den is enhanced by the presence of an adult capable of protecting them from snakes and other ground predators while the lactating females are left free to forage. Future field studies of other species of social mongooses may reveal that male guarding of the young is characteristic of this group. My observations on *Suricata suricatta* indicate that one or more adults including males typically stay with young at the den and Rasa (personal communication) reports that this also occurs in captive *Helogale undulata*.

Male guarding in the social mongooses has few parallels in other mammals. Male marmosets of the genera *Saguinus*, *Cebuella*, and *Callithrix* are reported to transport the young from the time they are born transferring them to the female only for nursing¹. Among social carnivores, wild dogs (*Lycaon pictus*) usually leave a guard at the den with the young when the pack hunts and this role is shared in turn by females and particular males²; in most cases, however, it is the lactating female which guards although one or more males may accompany her³.

TABLE 1 Guarding of young in three banded mongoose packs

	Males	Adults		Unsexed	Juveniles and subadults (< 1 yr)	Total
		Lactating females	Non-lactating females			
Pack composition:						
E pack	8	4	2	1		15
F pack	13	3	13			29
I pack	8	3	3		3	17
Total	29	10	18	1	3	61
No. marked	21	9	13		3	46
No. marked individuals which guarded	13	0	5		2	20
No. times marked animals acted as guards	35	0	10		3	48
No. times marked animals guarded alone	17	0	3		0	20

ing with the young in the morning three packs were live-trapped and individuals ear clipped and marked in distinctive patterns with Nyanzol fur dye. All mongooses in one pack were marked but it was not possible to trap all members of the other two packs. Mongoose packs ordinarily change dens every few days. When the den site of a marked breeding pack was known, I observed and recorded the total number of mongooses leaving each morning and the identities of those marked. Any marked animals staying in the den could then be determined by the process of elimination. On some occasions a guard emerged with the pack and then returned to the young when the others left. Usually only one mongoose stayed at the den (77% of 57 observations) but up to 6 guards were recorded. On three occasions all pack members went off leaving the young alone.

Table 1 gives the pack compositions and the guarding

This research was partially supported by a grant from the Uganda National Research Council.

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book reviews

Piaget on memory

Memory and Intelligence. By Jean Piaget and Bärbel Inhelder, in collaboration with Hermine Sinclair-de Zwart. Pp. xii+414. (Routledge and Kegan Paul: London, September 1973.) £5.25.

WITH the appearance of an English translation of this book there is now available to the English speaking world a full circle of Genevan writings that focus on particular topics of cognitive functioning.

In the introductory section of the book Piaget and his co-authors link up the words "memory" and "conservation". Memory is seen as an organism's capacity to conserve something over time; but is all conservation identical with what is called memory? Conservation could include here the entire genetic past inherited by an individual, as well as the modifications he acquires during his lifetime, many of which are conserved more or less indefinitely. Piaget is willing to speak of "memory in the biologist's sense" with an extremely broad meaning for memory which includes both physiological (acquired somatic responses) and behavioural modifications.

"Memory in the wide sense" includes conservation of behavioural habits together with recall of memory images and acts of recognition. Here, however, Piaget follows the logic of his own structural theory and makes two crucial points. First, recognition, if it means the recognition of perceptual signals, is an integral part of all perceptual or behavioural habits and must be clearly differentiated from a higher-level capacity to conserve and recall an image. Second, if conservation of habits (such as eye-hand coordination or spatial know-how) is ascribed to memory, then all further action-knowledge (and for Piaget knowledge at all levels is primarily action or 'operative' knowledge) must also be included in this memory. But an operative scheme, whether on a practical (sensori-motor) or theoretical (operator) level, is not something a person remembers but something he has or is. Thus a person with the operative scheme of, say, classification does not have to remember class inclusion but simply knows that—given that London is in England—there are more people in England than in London. This kind of knowledge (or understanding) is conceptualised as being based on a hierarchy of operative schemes (Piaget calls it "schematism"). These schemes are general instruments

of assimilation of incoming data and at the same time they are accommodated when applied to particular data.

Consequently the conservation of general assimilatory schemes that are integral and permanent belongings of the person must be distinguished from the conservation of particular accommodations that took place at a certain time during the person's past. The first kind of conservation is based on internal self-regulatory mechanisms that assure the scheme's permanent functioning, whereas only the second kind of conservation can be called "memory in the strict sense". Piaget's aim in this book is to investigate the relation between the first and second kind of conservation. This aim is implied in the book's title: *Memory and Intelligence*, where memory is taken in the strict sense and intelligence means the totality of schemes available to the child. These schemes develop in the course of the child's life experiences and the resulting changes from sensori-motor to pre-operative and finally to operator stages are reflected not merely in the child's operative structure of intelligence but also in his memory. For it is hypothesised that this operative structure is essential in the structuring and restructuring of input to be retained in memory.

Parallel with the distinction just outlined goes the contrast between the operative aspect of knowing which emphasises the actively transforming and assimilating character of knowledge and the figurative aspect which emphasises the accommodation pole of knowledge, namely the relatively static character of a particular sensory configuration inherent in any concrete object or event. A related distinction—important to a discussion of memory—is between the signifying and signified (or meaning) aspect of a sign. The various distinctions can be related to this topic by stating that memory in the strict sense emphasises the figurative aspect of knowing, that recognition is basically an undifferentiated sensori-motor signal reaction, while the memory response of reconstruction and recall is a differentiated signifying symbol. A memory image is sometimes called a schema—a simplified figurative model that serves as a mnemonic device—and must not be confused with the operative scheme on which the meaning aspect of a mental image is based. None of the above distinctions must be taken in an absolute or isolated sense: they are interrelated

aspects of a person's cognitive functioning.

Within this developmental and epistemological framework Piaget and his co-authors describe their programmatic research on memory. Twenty chapters cover investigations dealing with memory for additive logical structures, multiplicative logical structures, causal structures and spatial structures. For example, there is the report in chapter 4 on the remembrance of conflicting numerical and spatial correspondences. This conflict is brought about by the display of a straight line of four matches and below it, aligned from the left, another W-shaped line of four matches. Therefore, the child saw two lines which had the same number of identical sticks but differed in left-to-right extension. After a week and again after 6 months children were required to make a memory drawing. The responses of the children were classified into five types: 1, elimination of one line; 2, two lines of equal length, one straight, the other zig-zag, of undetermined number; 3, two lines of four matches with an attempt to equalise the boundaries; 4, two lines of an arbitrary number with the straight line correctly projecting; 5, correct in number of matches and length. Types 2 to 4 indicate different ways of solving the conflict between 'same number' and 'different length'. The children's memory solutions were clearly related to developmental age; types 2 to 4 were observed in many of the younger children after a week, but only in children from 7 years on after 6 months. The authors conclude that memory after 6 months, even more than more recent recall, is subordinate to the availability of operative schemes which in the present case meant co-ordination of apparently conflicting numerical (same) and spatial (different) correspondences.

English-speaking psychologists will probably find many places where the reporting is too global and qualitative or where the table of results or the criteria for scoring are not unequivocally defined. But these investigations can and should be critically scrutinised, evaluated, replicated and modified by the scientific community. Certainly lack of methodological finesse need not blind the reader to the substantive questions on memory with which these investigations are concerned. Although Piaget's style is far from being an example of clarity, it has an internal consistency which allows the motivated reader to recognise the un-

derlying thinking. But when a professional translator, apparently without an adequate understanding of Piaget's theory, goes to work and renders the author's cumbersome French into well formed English sentences, the result, as seen in this book, can be truly disastrous. The very first pages are so full of translation errors that comprehension of Piaget's position on the basis of this introduction is out of the question.

For example, the translator does not stick to the same words in translating the original words: memory, remembrance, recognition, recall, which are definitely not interchangeable. 'Imprinting' is a technical term used by the translator where 'registration' would have been more appropriate. The French 'ou' can mean an equivalence (remembrance = conservation) or a disjunctive alternative (remembrance of schemes or of data): the translation does not clarify this ambiguity. In page 3 paragraph 3, "Habit . . . concerns that part of the memory we call recognition", is the translation given for Piaget's "habit . . . includes recognitory memory". Where Piaget carefully distinguishes operational (in contrast to pre-operational) and operative (in contrast to figurative) the translation generally renders both words as operational; similarly, Piaget's distinction between an operative scheme and a figurative schema is largely confused.

After the introduction the reporting of the investigation is less theoretical and the translation is no longer replete with serious and confusing errors. Piaget's writings are certainly a problem, admitted by him and all, quite apart from translation. I hate to think of anything like an 'official' translation, yet this translation is not only inadequate but totally confusing as regards a potential comprehension of Piaget's psychological theory. With this proviso I recommend to all persons interested in Piaget's theory this important addition to his work on cognitive functioning and its development.

H. FURTH

Filling a nuclear gap

The Fundamental Particles. By B. H. Bransden, D. Evans and J. V. Major. Pp. vii+284. (Van Nostrand Reinhold: London and New York, September 1973.) £10.50.

THE continuing expansion of nuclear physics research and the increasing adoption of short unit lecture courses in physics departments has led to the splitting of topics within nuclear physics. This is undoubtedly good practice and the physics of fundamental particles is an obvious unit choice. This book, aimed at the undergraduate student, is well written and presented and should

serve its purpose as an introductory text. It contains a series of useful problems at the end of each chapter and is supplemented by some mathematical appendices on such topics as angular momentum, transition rates and the Weyl and Dirac equations.

After an introductory chapter, the authors concentrate for two chapters on the experimental methods of accelerating, transporting and detecting particles and measuring their masses and lifetimes. The treatment is selective but detailed. Then follow chapters on the conservation laws, symmetry principles and intrinsic quantum numbers. The pion-nucleon interaction is treated in detail using partial wave analysis. This chapter is very well put together. A chapter on mesons is followed by a discussion on the classification of particles including the quark model and the Regge classification. A description of high energy collisions follows. The authors conclude their book with chapters on electromagnetic properties and weak interactions.

The coverage is thus very extensive but the interest of the reader is well maintained throughout. The mathematics assumes some acquaintance with quantum mechanics. I find the book exceedingly readable. The introduction to each chapter is commendable. There is almost a complete absence of references in the text but a brief bibliography leads the reader onward.

This book adequately fills a gap between the simple and more advanced treatises on the subject, though its price is high.

P. R. BLAKE.

Astronomy updated

Astrophysical Quantities. By C. W. Allen. Third Edition. Pp. x+310. (Athlone), University of London. London. Distributed by Tiptree, Essex, November 1973.) £6.25.

THE eagerly awaited third edition of Professor Allen's invaluable reference book proves to be slightly disappointing. As far as updating the previous edition is concerned, Allen has done a good job, but the sections devoted to topics which have grown up since the early 1960s are less satisfactory. The "entirely new sections" include plasmas, solar wind, solar XUV, pulsars, cosmic X-rays, quasars and Seyfert galaxies. But the pulsars section consists merely of half a page defining the variables relevant to the study of these objects and a table giving the parameters of only thirteen pulsars; even three years ago a much more useful compilation of pulsar data were provided by Maran and Modali (*Earth and Extraterrestrial Sciences*, 1, 147; 1970), a source to which Allen does not even refer. It is a similar tale with

the other new sections.

The virtue of previous editions has been that they covered just about everything, removing the need to hoard articles such as that of Maran and Modali. But it is a remarkable achievement for one man to have produced the original version of *Astrophysical Quantities*, let alone ensured that the latest edition is still invaluable almost 20 years after the original appeared.

In his preface, Professor Allen suggests that the time to begin work on the fourth edition is now and asks for offers of help with its presentation. I hope that some dedicated practitioner of the new astronomy will indeed be willing to collaborate with Professor Allen on the daunting task ahead.

JOHN GRIBBIN

How behaviour develops

Behavioral Embryology. Edited by G. Gottlieb. Pp. xix+369. (Studies in the Development of Behavior and the Nervous System 1.) (Academic: New York and London, October 1973.) \$22.50.

THE first of the four sections in this book is an introduction to behavioural embryology, by G. Gottlieb. Then there are sections on embryonic motility and its neural correlates (Hamburger; Provine; Foelix and Oppenheim; Berrill); hatching: hormonal physiological and behavioural aspects (Oppenheim; Corner, Bakhuis and van Wingerden); and sensory processes: embryonic behaviour in birds (Vince; Impeken and Gold).

The subject is obviously of the first importance; few would quarrel with the idea that one of the best ways of approaching an understanding of adult form and function is to study their development. It is a pity that the misleading name 'behavioral embryology' has been adopted for what should be the embryology (or ontogeny) of behaviour.

The first two volumes of this series are intended to present a "detailed explication of the major 'philosophical', theoretical and empirical issues" involved. Since Gottlieb's introductory essay is presumably partly an attempt to explicate the philosophical, theoretical and empirical issues, it is somewhat disconcerting for the reader to come across an apparent confusion (pages 31, 32) between neural specificities and neural connections, which are not at all the same thing. Dr Gottlieb is in good company here, since this is a fairly widespread confusion. It does suggest, however, a certain laxity of approach; and this impression is not helped by a passing reference (page 25) to retinal cells connecting ("in a general way") to cells in the visual cortex.

An almost mystical element is introduced in some of the arguments in Hamburger's paper on the anatomical and physiological basis of embryonic motility in birds and mammals. Hamburger draws attention to the incongruity between neurogenesis and overt motility in amniote embryos. The unorganised movements of the 4-17-d-old chick do not, apparently, reflect the neurogenetic events going on, so to speak, below the surface. From these observations Hamburger claims that it is not valid to generalise that neurogenesis fully explains behaviour. And he makes the remarkable statement that "even the most detailed knowledge of neural organisation, including all significant synapses, in chick or rat embryos at a given stage would permit no prediction of the actual (type 1) movements performed at this stage". This viewpoint must command respect; but it surely does so as a statement of faith, of scientific position, rather than as a rigorous argument from observed data. It cannot be the latter, of course, since too many of the premises are hidden: as Hamburger points out, the observations on movement are crude and the information available on structure and ultrastructure is deplorably inadequate. After further arguments involving the results of transplanting parts of the embryonic spinal cord, Hamburger comes to the conclusion, with which I agree, that the conceptual dichotomy of coordinated against uncoordinated movements is perhaps too rigid and that such dichotomous thinking should give way to a more flexible scheme.

This book is interesting and contains much relevant information. Even arguments with which individual readers may disagree are worthwhile if they stimulate thought on this subject, where thought is badly needed.

R. M. GAZE

Computed structures

Molecular Structures and Dimensions, Vol. A1: Interatomic Distances 1960-65: Organic and Organometallic Crystal Structures. Edited by Olga Kennard, D. G. Watson, F. H. Allen, N. W. Isaacs, W. D. S. Motherwell, R. C. Pettersen, and W. G. Town. (N.V.A. Oosthoek's Uitgevers Mij: Utrecht; distributed by Crystallographic Data Centre, Cambridge and Polycrystal Book Service, Pittsburgh, 1972.) n.p.

To discover the structures of molecules has been one of the main aims of chemistry since the science began. To discover the dimensions of these molecules only became possible with the advent of X-ray crystallography and various spectroscopic methods. By 1960 a very

large amount of information had been accumulated on interatomic distances and the configurations of molecules and ions. L. E. Sutton was a pioneer in the compilation of all this data, a task continued by the Chemical Society in its Special Publications.

About 1960, however, a profound change began to take place in the methods of X-ray crystallography. Fast computers and automatic diffractometers became available. The collection and analysis of X-ray measurements were vastly speeded up, and the accuracy of structure determinations greatly increased. About this time it began to be realised that the structure of an unknown molecule like a natural product could be determined more quickly and with greater certainty by the methods of X-ray crystallography than by the classical methods of organic chemistry. All this has led to a spectacular increase in the number of structure determinations from the early 1960s onwards. Up to 1972 about 8,500 organic and organometallic structures had been published, and each structure usually contains a very large amount of accurate numerical data. This output is now far beyond the capacity of the ordinary textbook to handle. But the computers that created the problem have now come to the rescue.

The Crystallographic Data Centre in Cambridge, with the sponsorship of the Office for Scientific and Technical Information have a computerised data file which contains all this information and to which about 1,500 new entries are added annually. Annual bibliographic volumes are published. The present volume is the first to give all the numerical data for organic and organometallic structures, and covers the years 1960-65. Although it modestly claims to present interatomic distances, as a continuation of Sutton's work, it does in fact cover much more than this. In addition to the chemical structural formula each structure is illustrated by a beautifully drawn stereoscopic pair (viewer provided) which has been computer generated from the published atomic coordinates. The numerical data give bond lengths, bond angles and torsion angles. These are all recomputed from the published coordinates and discrepancies noted. The torsion angles, which provide vital information concerning molecular conformation, were seldom given in papers published between 1960 and 1965 and so this volume contains a vast mine of new information. Another valuable feature is the inclusion of the *R*-factor for each structure, giving an indication of its reliability. It is interesting to note that for the 791 full three-dimensional structures listed, the *R* values range from 0.02 to 0.29, with a mean

value of about 0.10. A value of about 0.06 indicates a very accurate analysis, while a value greater than about 0.15 indicates the need for further refinement in most cases, unless the structure is extremely complex with some disorder.

The layout of the whole volume is excellent, but unfortunately it is not easy to read all the atom numbers on a few of the more complex diagrams, such as for vitamin B₁₂ and epilimonol iodoacetate. Those whose eyes do not adapt easily to stereo vision might have preferred a single larger diagram. But on the whole this is a magnificent production and Olga Kennard and her co-editors must be congratulated on their achievement. I hope and trust this heroic task will be continued with the future volumes that are contemplated. All scientists interested in molecular structure are greatly in their debt.

J. MONTEATH ROBERTSON

How to be a petrologist

Experimental Petrology: Basic Principles and Techniques. By Alan Edgar. Pp. xi + 217. (Clarendon: Oxford; Oxford University: London, October 1973) £5.75.

Books written for research workers are, in overwhelming majority, about what to think and do; they represent an attempt on behalf of the author to fashion the way his readers will approach their research problems. In contrast, this book sets out to describe how to do something: in this case, experimental petrology. It is unashamedly a recipe book destined to be fingerprinted with molybdenum and bleached with spots of dilute nitric acid.

In fulfilling this aim, the author has described clearly and accurately the anatomy and function of the sort of apparatus a new researcher might expect to find in any established laboratory of experimental petrology. This book will answer in advance many of the questions such a person would need to ask during their first months in the field. But the aims seem to go deeper than this as there is a quantity of detailed information about the essentially engineering side of the subject which would only be of concern to a person setting up a laboratory from scratch. I doubt whether it is realistic to hope that any book could include enough of this sort of detail to be fool-proof but at least the present work gives a reasonable guide to the literature and compares different techniques (for example starting materials) in a sensible and unbiased manner.

The level of theoretical background given is uneven. Buffers for controlling

the chemical potential of volatile species are dealt with in some detail (although I was surprised to find no reference to the work either of Burnham, Holloway and Davis or of Presnall on the *PVT* of water and hydrogen respectively). On the other hand, the treatment of one, two, and three component systems in chapter 2 is very cryptic; for example, the free energy of binary solutions receives less than one page and will surely convince most non-initiate readers that there is a large element of magic in thermodynamics.

But this book's great merit is that it does what it sets out to do and at £5.75 represents a worthwhile investment to anyone about to embark on research in the field of experimental petrology.

S. W. RICHARDSON

Life on Moon and Mars

Origins of Life: Planetary Astronomy. Edited by Lynn Margulis. (Proceedings of the 3rd Interdisciplinary Communications Program Conference.) Pp. xi+268. (Springer-Verlag: Berlin and New York, 1973.) DM46.70; \$16.50.

THIS is a verbatim report of the third of four conferences on the origins of life. The style of the volume follows that of its predecessors (see *Nature*, **235**, 404; 1972; and **239**, 175; 1972). The publisher, however, has changed, and the price has come down by over 40%. The conference, too, was organised differently. On this occasion the purpose was to explore the effect of space exploration on ideas about the origins of life. For the purpose, eight planetary astronomers, who were personally involved in space programmes then current, were assembled to meet ten practitioners interested in the origins of life (three exobiologists, two microbiologists, four chemical prebiologists and one palaeontologist). A fair proportion of the biologists had attended at least one of the previous conferences, but most of the astronomers were there for the first time. The conference was held in February 1970.

There was not the same 'free for all' that characterised the earlier conferences. On the contrary, there was a rigid division into two halves, the first devoted to the Moon, the second to Mars. In each division, certain of the astronomers were asked to discourse on aspects of their topic that they thought might interest the biologists. The biologists were encouraged to interrupt, and to ask questions. During the first division, Shoemaker, Wasserman, Kaplan and Singer discoursed on the Moon; then Oró went into considerable detail on the chemical search for carbon compounds in lunar material, and Young went into the methods

adopted in the search for microbiological activity. In the second half, Murray, Owen and Leovy spoke about Mars. Some biological interest was aroused in the part that nitrogen might or might not play in life on the planet.

But the book, if not the conference, seems to have failed in its objectives. The timing was unfortunate. Knowledge of Mars has changed dramatically with the new data collected by Mariner 9 in the following year, and the review of the Moon (which, in any case, is of less biological interest than Mars) was based on data emanating from nothing later than Apollo 11. There has been a three-year delay in publication, and the field is just moving too fast for such a leisurely programme. The conference lacked the enthusiasm of its predecessors. The book is not only out of date; it is dull. Better accounts of both the astronomy and geology can be found elsewhere. It is especially to be regretted that no attempt was made to reproduce the illustrations of lunar and Martian topography which, from all accounts, formed a major feature of the conference.

P. C. SYLVESTER-BRADLEY

Well digested Protozoa

The Biology of Protozoa. By M. Sleight. Pp. viii+315 (Arnold: London, October 1973.) £7.50 boards; £3.75 paper.

RECENT spectacular increases in knowledge of the genetics, biochemistry and ultrastructure of the Protozoa have greatly increased the difficulty of writing a satisfactory book of modest size on this group. Dr Sleight has attempted to produce in 300 pages, at about degree level, a general biology of the Protozoa, for the most part successfully.

After a brief introduction on evolutionary relationships and cellularity, three chapters are concerned with structure, metabolism and reproduction. Of the remaining seven chapters, six are devoted to taxonomic groups. It seems to me regrettable that the general treatment with which the book begins (and ends: chapter 11, "Ecology of Protozoa") was not carried through completely. But repetition, which is liable to occur in combining a systematic with a subject approach, is largely avoided by numerous cross references. Complex ascriptions in the text are minimised by the numerical reference system; hence the presentation is one of digested rather than of quoted information. Occasionally one could wish for more references to the sources of information, as in Table 5.1 of "Features of Various Flagellate Groups".

The illustrations are of diverse kinds, light and electron micrographs, draw-

ings and diagrams and are generally fresh and apposite although some of the drawings of whole animals made them look, to me, more solid and opaque than protozoa usually do in the microscope.

This is altogether a valuable and timely book of the right size for use in undergraduate courses.

G. CHAPMAN

Mediterranean collection

The Mediterranean Sea: A Natural Sedimentation Laboratory. By D. J. Stanley. Pp. xvi+765. (Dowden, Hutchinson and Ross: Pennsylvania; Wiley: Chichester, September 1973.) £22.50.

THIS volume is a collection of forty-seven papers brought together out of the feeling that the Mediterranean Sea is an ideal environment for the study of marine sedimentation, and in the belief that sedimentological work in this area, cutting across linguistic and national boundaries, should be summarised in order to speed future investigations. The papers are divided between twelve sections touching on many different aspects of the Mediterranean, its bathymetry and hydrography, the geological setting up to and including the Quaternary, clastic and non-clastic sedimentation on shelves and in coastal areas, deep-water sedimentation, and geochemistry. There is a paper on pollution of the Mediterranean and another sketching which topics should be given priority in research in the next decade.

Although a commendably high editorial standard is apparent, the content and relevance of the papers are uneven and not wholly representative of recent work in the Mediterranean. Some papers are regional or subregional in scope, whereas many deal with what could be purely local problems, and a few simply use the Mediterranean as the setting for the study of a general topic. Among these personal original endeavours, I sorely missed attempts at regional syntheses specific to the Mediterranean, which would aim to show in detail what was known and what remained to be studied. It is easy from this book to discover fairly completely who is doing what and how—it is excellent as a compilation transcending national differences in scientific outlook—but I am less confident that a critical light has been focussed on the major research problems of the Mediterranean and its hinterlands. A different blend of original papers and critical reviews might perhaps have led to a book greater than the sum of its parts.

J. R. L. ALLEN

matters arising

Evolution of the Tasman Sea

SIR,—Hayes and Ringis¹ have presented an interesting model for the evolution of the Tasman Sea. I wish to call attention, however, to what I feel is an inappropriate reference to an earlier article by myself and Brennan². Hayes and Ringis¹ state that: "A cursory examination of widely spaced east-west aeromagnetic profiles between Australia and the Lord Howe Rise led to the early but incorrect conclusion that there were no lineated magnetic anomalies within the Tasman Sea." But in discussing the possibility of lineated anomalies in Tasman Sea we stated that: "While the lack of correlated north-south anomalies cannot be proved conclusively from our data, if such correlation does exist it is not apparent."

Furthermore we said: "But the fact that the individually widely spaced magnetic profiles resemble magnetic profiles produced by the process of seafloor spreading means that the possibility that these magnetic anomalies were produced by an earlier episode of seafloor spreading cannot be excluded. This earlier period of seafloor spreading could have been complex, requiring a closely spaced survey to detect its presence." From our study we concluded that: "A more detailed survey should be conducted to resolve these two contradictory ideas (that is, lineated as compared with nonlineated anomalies) concerning the source of the anomalous magnetic pattern formed in the Tasman Sea."

Unfortunately, some confusion may result from the title given to our letter

on the index page of the issue in which it appeared. It was referred to as: "No seafloor spreading in the Tasman Sea," perhaps an unfortunate summary in view of the above.

My present comment does not, of course, effect the substance of the Hayes and Ringis paper but merely seeks to clarify the interpretation of an earlier reference.

Yours faithfully,
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¹ Hayes, D. E., and Ringis, J., *Nature*, **243**, 454 (1973).

² Taylor, P. T., and Brennan, J. A., *Nature*, **224**, 1100 (1969).

science on television

Under the BBC's bushel

John Gribbin and Fiona Selkirk

IF you ask the average scientist in the street (let alone the apocryphal man on the Clapham omnibus) what science programmes are to be found on British television he would probably reply "Well, there's 'Horizon', of course, and the Open University, and I think there was a programme about anorexia on ITV the other day, and sometimes odd bits late at night on BBC." In fact, those odd bits amount to regular broadcasts in 30 weeks of the year (excluding repeats) under the guise of further education. But the BBC in general, and *Radio Times* in particular, is so coy about this output that it is difficult to find what is on, and when, at the best of times. Recently, these programmes have suffered because of restrictions on late night television (in Britain that means after 2230) and because of the subsequent domination of our screens by election programmes; so these difficulties have been greatly enhanced. We therefore found it necessary to resort to a visit to the BBC Tele-

vision Centre, where several hours spent in a viewing room provided the material needed to comment on this almost undercover output from the BBC.

Of the forthcoming attractions "The Experimenters", provisionally scheduled to be shown on BBC1 on Mondays from April 22, will probably be of considerable interest to a scientifically knowledgeable viewer.

It is seldom possible to combine scientific research with vocational work and the choice after graduation is a difficult one to make and even more difficult to go back on if the choice transpires to have been wrong. A doctor in a mining community in South Wales has, however, managed to do just this and plans to provide some useful information about the occurrence and development of gut cancer, while still practising medicine in the community. He is the 'experimenter' in one of these programmes. The series attempts, and if this example is representative, succeeds, in looking at the people behind scientific research, their methods and motives.

This doctor tried pure research and decided he wanted to know his 'guinea pigs' as people. He took up general

practice and is starting a survey into gut cancer using his patients as the subjects.

The scene of his meeting with the local community in one of the local clubs—on Bingo night—is effective. The attentive, slightly worried faces of his audience are intriguing as he explains what he is trying to do. "Why is this survey being done here and not in London?" he was asked. With a wry smile, he explained about population stability and community spirit, features not exactly characteristic of the population of an average big city.

There are problems involved with doing this kind of research, the biggest being communication, but this doctor argued convincingly that the feedback from research improves his value as a general practitioner even if the research turns out not to be of any great significance in itself.

Even in the laboratory, however, the halcyon days of scientific breakthroughs being made by a lone researcher are gone and today scientists have to work in teams. The choice of team mates is critical or research can grind to a halt through personality differences, even though the minds behind those per-

sonalities may have the same goal.

This concept was well illustrated by the differing attitudes to the controversial results of some research by a team of three more 'experimenters' in the series.

Drs Walter Kellerman, Gordon Brooke and John Baruch are now famous as the team that might have discovered a quark, or not, as the case may be. Baruch is brashly convinced of the validity of their cosmic-ray experiments, and hustled his colleagues into publication. "Why let someone else get the credit?", he argued. Brooke seemingly regrets this haste and says that he would not have published this claim without more evidence, if he had had his way. And Kellerman, balancing these extreme views and presumably with the casting vote, decided in favour of some publicity, to encourage further work in the field (and, no doubt, to encourage further grants to the group at the University of Leeds).

The discussions between the members of the team certainly showed the human face of science (with a few small warts); and Kellerman was heretical enough to point out that the 'scientific method' is a mythical beast.

Two other programmes came from series already screened and perhaps even seen by those people who were able to find out that they were on. A look at some of the 20,000 or so tiny creatures that share the average house with its

human occupants was not for the squeamish adult but, overall, with light-hearted narration, a remarkable range of background music and some expert photographic work, it was just the stuff to give an appreciative audience rather earlier than 2325—although perhaps not just after supper.

The combination of some of the best photographic techniques (the use of light and scanning electron microscopy, time-lapse and excellent closeup camera work) makes this extremely successful visually, rivalling the best of "Horizon", "The World About Us" and others.

As for "Bellamy on Botany", Dr David Bellamy has, through the force of his own personality, ensured that his contributions to the further education output are reaching a wide audience. Bellamy has the character and personality to carry a programme transmitted live from a sewer (perhaps this will come in due course), but the programme we saw came from the clean dunes of Holland; we learned how natural reclamation of land from the sea takes place as plants establish stable dune formations.

The comparison with programmes such as "Horizon" is again favourable—especially so since, as we were told, a number of further education programmes can be produced for the cost of one "Horizon". These programmes may be labelled education, and that may put off some casual viewers, but as we saw they can be as hard hitting as any cur-

rent affairs programme, or as entertaining as any feature, as well as being educational.

And what of programmes aimed specifically at schools? One silver lining to the recent rail travel troubles in southern England has been the opportunity to view some of these, along with several thousand schoolchildren and an unknown number of housewives. On the basis of this limited opportunity, the scientific programmes for schools, especially those for 16 to 18-year-olds, are every bit as good as their late night counterparts. Indeed, many of them could be shown unchanged in the evenings, and it is rather odd that although further education programmes are sometimes repeated for schools, the reverse flow is non-existent. Here, surely, is a useful way to occupy the 22 weeks when further education programmes disappear from their usual slot.

But one thing is certainly clear. There is no need to worry about the health of science on television in Britain; it is alive and well, and living under the name of "further education". What is worrying is that these programmes receive little or no promotion, and that those responsible for screening and publicising the programmes seemingly lack the imagination of the people who produce them. Until this situation is improved, a little research into the small print of the *Radio Times* is likely to prove a very rewarding exercise.

Announcements

Erratum

In the article 'Lyoluminescent tissue equivalent radiation dosimeter' by Nadir A. Atari and Kamil V. Ettinger (*Nature*, 247, 193; 1974) there is a misprint in the title: 'dosimeter' appeared as 'desimeter'. Also paragraph 6, line 10 should read ... 'between 1 and 10⁶ rad ...' not ... 'between 1 and 60⁶ rad ...'.

Reports and Publications

not included in the *Monthly Books Supplement*

Great Britain and Ireland

- Science Research Council—Electrical and Systems Engineering Committee. Report on Electrical Machines. Pp. iv+19. (London: Science Research Council, State House, High Holborn, 1973.) 240p. [2410]
- Department of the Environment. First Report on Research and Development 1973. Pp. vi+56. (London: HMSO, 1973.) 42p net. [2410]
- The Mathilda and Terence Kennedy Institute of Rheumatology. Sixth Annual Report. Pp. 67. (London: The Mathilda and Terence Kennedy Institute of Rheumatology, 1973.) [2510]
- Department of Education and Science. Safety in Science Laboratories. (DES Safety Series, No. 2). Pp. iv+39. 32p. Safety in Practical Departments. (DES Safety Series, No. 3). Pp. iv+43. 32p. Safety in Physical Education. (DES Safety Series, No. 4). Pp. iii+22. 21p. (London: HMSO, 1973.) [2610]

Final Report of the Anti-Locust Research Centre, 1 January 1970–31 May 1971. Pp. 72. Centre for Pest Research—Descriptive Brochure. Pp. 24. Centre for Overseas Pest Research—Report June 1971–December 1972. Pp. 148. (London: Centre for Overseas Pest Research, 1973.) [2610]

Other Countries

- Scanning Electron Microscopy of Asporic Aspergilli. By R. Locci. (Supplemento al Vol. VIII, Serie IV, 1972 della Rivista di Patologia Vegetale.) Pp. 172. (Milano: Istituto di Patologia Vegetale, Cattedra di Micologia, Via Celoria 2, 1973.) [2210]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 72-12: Subsurface Lower Paleozoic Stratigraphy in Northern and Central Alberta. By D. C. Pugh. Pp. iii+54. \$5. Paper 72-47: Geology of Tavani Map-Area, District of Kewatin. Pp. iii+14. \$1.50. Paper 73-19: Paleomagnetic Results from the Tertiary Mount Barr and Hope Plutonic Complexes, British Columbia. Unit Correlations and Tectonic Rotation from Paleomagnetism of the Triassic Copper Mountain Intrusions, British Columbia. By D. T. A. Symons. A Ballistic Magnetometer for the Measurement of Rock Magnetic Properties. By E. J. Schwarz and T. Whillans. Pp. iv+34. \$2. Paper 73-21: Field and Laboratory Methods used by the Geological Survey of Canada in Geochemical Surveys. No. 12: Mercury in Ores, Rocks, Soils, Sediments and Water. By I. R. Jonasson, J. J. Lynch and L. J. Tripp. Pp. iii+22. \$2. Paper 73-29: A Grenville Front Magnetic Anomaly in the Megiscane Lake Area, Quebec. By B. W. Charbonneau. Pp. iii+20. \$2. (Ottawa: Information Canada, 1973.) [2210]
- Lawrence Berkeley Laboratory. Research Highlights 1972. Pp. 60. (Berkeley: Lawrence Berkeley Laboratory, University of California, 1973.) [2210]
- CERN—European Organization for Nuclear Research. CERN 73-9: Diagrammar. G. 't Hooft and M. Veltman. Pp. iv+114. (Geneva: CERN, 1973.) [2210]
- Smithsonian Contributions to Zoology, No. 147: The Old World Stenodidae: a Preliminary Survey of the Fauna. Notes on Relationships, and Revision of the Genus *Eriogenes* (Lepidoptera: Gelechioidea). By W. Donald Duckworth. Pp. 21. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) 40 cents. [2210]
- United States Department of the Interior: Geological Survey. Professional Paper 764: Stratigraphy

- of the Southern Coast Ranges near the San Andreas Fault from Cholame to Maricopa, California. By T. W. Dibblee, Jr. Pp. iv+45. 80 cents. [2210]
- Geological Paper 765: The Oxidized Uranium Ore Deposits of the Tordilla Hill-Deweesville Area, Karnes County, Texas: a Study of a District Before Mining. By C. M. Bunker and J. A. Mackallor. Pp. iii+37. Professional Paper 775: Petrography of Some Granitic Bodies in the Northern White Mountains, California-Nevada. By Dwight F. Crowder and Donald C. Ross. Pp. vi+27. 70 cents. (Washington, DC: Government Printing Office, 1973.) [2210]
- United States Department of the Interior: Geological Survey. Professional Paper 747: Pennsylvanian Carbonates, Paleogeology, and Rugose Colonial Corals, North Flank, Eastern Brooks Range, Arctic Alaska. By Augustus K. Armstrong. Pp. v+21+8 plates. (Washington, DC: Government Printing Office, 1972.) \$1. [2310]
- US Department of Health, Education and Welfare. National Institutes of Health. DHEW Publication No. (NIH) 74-575: Soviet Medicine—a Bibliography of Bibliographies. (A Publication of the Geographic Health Studies Program of the John E. Fogarty International Center for Advanced Study in the Health Sciences.) Pp. vii+46. (Washington, DC: Government Printing Office, 1973.) 80 cents. [2310]
- Methods for the Analysis of Human Chromosome Aberrations. Edited by K. E. Buckton and H. J. Evans. Pp. 66. (Geneva: WHO; London: HMSO, 1973.) 12 Sw. francs; £1.50; \$3.60. [2410]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 219: Lower Cretaceous Bullhead Group Between Bullmoose Mountain and Teetsa River, Rocky Mountain Foothills, Northeastern British Columbia. By D. F. Stott. Pp. 228 (15 plates). \$6. Bulletin 222: Contributions to Canadian Paleontology. By D. E. Jackson, B. S. Norford, D. S. Broad, D. L. Dineley, A. E. H. Pedder, A. R. Ormiston and L. Cameron Mosher. Pp. 192. \$6. Paper 73-11: Publications on the Geology of the Arctic Islands by the Geological Survey of Canada. Compiled by R. L. Christie. Pp. 39. \$1. Paper 73-36: Studies in "Standard Samples" of Silicate Rocks and Minerals, Part 3: 1973 Extension and Revision of "Usable" Values. By Sydney Abbey. Pp. 25. \$1. (Ottawa: Information Canada, 1973.) [2510]
- Smithsonian Contributions to Zoology, No. 152: Evolution of the Rails of the South Atlantic Islands (Aves: Rallidae). By Storrs L. Olson. Pp. iii+53 (11 plates). (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) 95 cents. [2510]